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LOW DOSE OF GAMMA IRRADIATION ENHANCED DROUGHT TOLERANCE IN SOYBEAN

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Drought stress is the main limiting factor in soybean production. However, no work has been done on how the application of a low dose of gamma rays could help to overcome water deficits during critical stages of soybean development. Gamma rays at a dose of 20 Gray (Gy) were applied to dry seeds of soybean before planting. Two levels of soil moisture (80% field capacity for well-watered control and 35% for drought-stressed treatment) were applied at pod initiation. Gamma irradiation increased biomass accumulation and seed yield in both treatments. It also increased the chlorophyll content, photosynthetic activity ($^{14}CO_2$ fixation) and leaf water potential and enhanced the enzyme activities of RuBPCase and PEPCase of control plants compared with drought-stressed plants. Gamma irradiation (20 Gy) increased the soluble sugars, protein and proline content and the activities of peroxidase and superoxide dismutase in drought-stressed soybean leaves. It also increased the chloroplast size, which was reduced by drought treatment, and rebuilt, to some extent, the chloroplast ultrastructure. However, it decreased the malondialdehyde concentration and the electrical conductivity of the leaves under drought stress. Overall, the results indicated that pre-treatment with gamma rays (20 Gy) to dry seeds of soybean before planting could be used to enhance drought tolerance and minimize the yield loss caused by water deficit.

Key words: antioxidative enzymes, drought stress, gamma irradiation, proline, soybean

Abbreviations: MDA: malondialdehyde; POD: peroxidase; SOD: superoxide dismutases; ROS: reactive oxygen species; H_2O_2: hydrogen peroxide; Ψ_{leaf}: leaf water potential; RuBPCase: ribulose-1,5-bisphosphate carboxylase/oxygenase; PEPCase: phosphoenol pyruvate carboxylase; Gy: Gray

Introduction

Soybean is one of the most economical and nutritious foods, which may be of help in countering malnutrition and undernutrition in developing countries. Drought limits plant growth on a large proportion of the world's agricultural land. Soybean is considered sensitive to drought stress, especially during critical

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periods of plant development (Liu et al., 2004). Water stress results in yield reduction by decreasing the seed number and seed weight. Intermittent drought is almost certain to occur during soybean ontogeny (Dornbos et al., 1989). Drought stress is the primary constraint for increasing soybean yield, particularly when it triggers an early switch from vegetative to reproductive development (Desclaux and Roumet, 1996). Drought is an important environmental factor, which induces significant alterations in plant physiology and biochemistry. The most common symptom of water stress injury is the inhibition of growth, which is reflected in a reduction in the dry matter yield (Le Thiec and Manninen, 2003). Water deficit inhibits photosynthesis as it causes alterations in chlorophyll content and harms the photosynthetic apparatus (Dalla Costa et al., 1997). In addition, it modifies the activity of some enzymes and the accumulation of sugars and proteins in the plant (Gong et al., 2005), resulting in lower plant growth and yield (Dalla Costa et al., 1997). Drought stress was found to decrease the relative water content of plant leaves (Sánchez-Blanco et al., 2002) and total chlorophyll (Shaddad and El-Tayeb, 1990) and to increase the accumulation of H_2O_2, lipid peroxidation, soluble proteins and free amino acids, including proline, in various plants (Gunes et al., 2008). Drought induces the generation of reactive oxygen species (ROS), causing lipid peroxidation, and consequently membrane injury, protein degradation, enzyme inactivation and the disruption of DNA strands (Becana et al., 1998). The MDA content is often used as an indicator of the extent of lipid peroxidation resulting from oxidative stress (Smirnoff, 1993). Drought stress may lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of reactive oxygen species, which are responsible for various types of damage to macromolecules and induce oxidative stress (Reddy et al., 2004). The reduced activity of RuBPCase, induced by biotic and abiotic stresses, is well documented in plants (Allen and Ort, 2001).

Gamma rays have proved to be economical and effective as compared to other ionizing radiations because of their easy availability and power of penetration. This penetration power of gamma rays helps in its wider application for the improvement of various plant species (Moussa, 2006). Sjodin (1962) reported that the material and energy necessary for initial growth are already available in the seed, so the young embryo has no need to form new substances, but only to activate those already stored in the cotyledons. Low doses of γ-radiation may increase the enzymatic activation and awakening of the young embryo, which results in stimulating the rate of cell division and affects not only germination, but also vegetative growth and flowering. Exposing the dry seeds to low γ-irradiation doses resulted in increased yields in some plants, such as sunflower (Abo-Hegazi et al., 1988) and *Ammi visnage* (El-Shafie et al., 1993). Also, Patskevich (1961) came to the conclusion that the irradiation of seeds prior to sowing held great promise from the viewpoint of its practical application in agriculture. It was generally agreed that low doses of gamma rays stimulate the cell division, growth and development of various organisms, including animals and plants. This phenomenon, named hormesis, was analysed and discussed by

various authors for various species (Korystov and Narimanov, 1997). Very low doses of gamma irradiation have been shown to stimulate plant growth (Watanabe et al., 2000). Previous studies have shown that the effects of relatively low doses of ionizing irradiation on plants and photosynthetic microorganisms are manifested as accelerated cell proliferation and increased germination rate, cell growth, enzyme activity, stress resistance and crop yields (Chakravarty and Sen, 2001). The objective of this work was to investigate whether pre-treatment of dry seeds of soybean plants with a low dose of gamma rays (20 Gy) before planting could act as a protective agent to nullify the influence of drought stress.

Materials and methods

Plant material, growth conditions and stress treatments

A homogeneous lot of soybean seeds (*Glycine max* L.), cv. Giza 83, was obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt and kept at 4°C. After surface sterilization in 0.1% (w/v) sodium dodecyl sulphate solution they were thoroughly rinsed with sterile deionized water. Dry seeds were exposed to 0.0 and 20 Gy doses of gamma irradiation using a gamma source (^{60}Co) (Vinderen, Oslo, Norway) at the Middle Eastern Regional Radioisotope Center for the Arab Countries (Dokki, Cairo, Egypt) with a strength of 500 Ci and a dose rate of 0.54 Gy/min. The seeds were germinated in pots (35 cm high × 30 cm diameter), each filled with 15 kg sandy loam soil with 2.5% organic matter and available N, P and K concentrations of 170, 80 and 200 mg kg^{-1}, respectively. The pots were arranged in a completely randomized design with two factors: two gamma irradiation doses (0.0 and 20 Gy) and two soil water levels (well-watered and drought-stressed), with 20 pots per treatment, replicated four times. The 320 pots for the experiment were placed in a field sheltered from rain by a removable polyethylene shelter, at a day/night temperature of 24/18°C, with 70% relative humidity, 14-h light and a photon flux density of 400 μmol m^{-2} s^{-1}. Cultural practices, such as weed control and irrigation, were performed as needed. Ten seeds were sown per pot. After the seedlings reached the first true leaf stage, they were thinned to four plants per pot. Two levels of soil moisture were applied by controlled watering beginning at pod initiation until harvest at full maturity. The well-watered and drought-stressed treatments were maintained at 80% and 35% soil field capacity, respectively, following the methods of Desclaux and Roumet (1996). The water deficit was initiated by withholding water. The pots were weighed daily to maintain the desired soil water levels by adding appropriate volumes of water. All biochemical estimations were carried out using three leaflets per newly expanded trifoliolate leaf. Samples were collected 10 days after the water treatment was applied, between 9.30 and 10.30 a.m., and kept in liquid nitrogen until analysed. The effect of the treatments on growth and yield was determined by measuring the accumulated biomass of the various organs. At harvest, the plants were removed carefully from the pots. The biomass and seed weights were determined after drying the harvested organs for 48 h at 70°C.

Enzyme assay

Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBPCase, EC 4.1.1.39) was determined as suggested by Warren et al. (2000), peroxidase (POD, EC 1.11.1.7) following the method of Macheix and Quessada (1984), superoxide dismutases (SOD, EC 1.15.1.1) as described by Dhindsa et al. (1981), and the activity of phosphoenol pyruvate carboxylase (PEPCase, EC 4.1.1.31) as described by Gonzalez et al. (1998).

Chemical analysis

Total soluble protein contents were measured using the method of Bradford (1976) and free proline according to the method described by Bates et al. (1973). Lipid peroxidation was measured in terms of malondialdehyde content using the thiobarbituric acid reaction, as described by Madhava Rao and Sresty (2000). Soluble sugars were evaluated using the anthrone method described by Fales (1951) and electrical conductivity using a digital conductivity meter (JENWAY, Model 4070, Essex, England). Leaf water potential (Ψ_{leaf}) was measured in a pressure chamber (Model 3000, Soil Moisture Equipment Corp, Santa Barbara, CA, USA).

Total chlorophyll

The total chlorophyll content of fresh leaves was estimated following the method suggested by Barnes et al. (1992).

Photosynthetic activity ($^{14}CO_2$ fixation)

Photosynthetic activity was measured in the Radioisotope Department of the Atomic Energy Authority, Cairo, Egypt, with the method of Moussa (2008). The seedlings from each treatment were placed under a Bell jar, which was used as a photosynthetic chamber. Radioactive $^{14}CO_2$ was generated inside the chamber by a reaction between 10% HCl and 50 μCi (1.87×10^6 Bq) $NaH^{14}CO_3$ + 100 mg Na_2CO_3 as a carrier. Then the samples were illuminated with a tungsten lamp. After 30 min exposure time, the leaves were quickly detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction with 80% hot ethanol. The ^{14}C was assayed from the ethanolic extracts in soluble compounds using a Bray Cocktail (Bray, 1960) and a Liquid Scintillation Counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises, Edinburgh, UK).

Isolation of chloroplasts

Chloroplasts were isolated from fresh leaves in chloroplast isolation buffer containing 50 mM Tris-HCl, 5 mM EDTA, 0.33 M sorbitol, pH 7.5 using the method of Block et al. (1983). Crude chloroplasts were purified by centrifugation using a 40%/80% Percoll gradient (Schwertner and Biale, 1973). Intact chloroplasts were collected from the gradients, diluted three to four times, and centrifuged at 2070 g for 2 min. They were then resuspended in the isolation buffer and kept in darkness until further use. All procedures were carried out at 0–4°C.

Electron transmission microscopy

For microscope observations, the lower epidermis was stripped off the leaves. Samples were prepared as described by Coulomb et al. (1996). Briefly, after fixation in glutaraldehyde and post-fixation in osmium tetroxide, they were dehydrated in acetone and embedded in araldite. The sections, stained in uranyl acetate and lead citrate, were examined by transmission electron microscopy (TEM, Jeol Jem 1200 EX II, Tokyo, Japan).

Chloroplast size determination

Chloroplast size distribution was determined with the dynamic light scattering technique (Beckmann, Coulter N4 Plus apparatus) at a scattering angle of 90°. A unimodal distribution was assumed for the mean particle size calculation.

Statistical analysis

All data were subjected to ANOVA and the means were compared using Duncan's multiple range tests ($P<0.05$).

Results

The average size of chloroplasts isolated from control soybean seedlings was about 1,200 nm and was not noticeably different from that of chloroplasts obtained from plants pretreated with 20 Gy gamma irradiation (Fig. 1). The chloroplasts obtained from drought-stressed plants were not much more than half this size. The decrease in average chloroplast size was less drastic in plants irradiated with gamma rays (20 Gy).

Transmission electron microscopy of the chloroplasts of untreated soybean seedlings revealed the typical ultrastructure, with a well-organized envelope and internal membrane structure with normally developed grana and stroma thylakoids (Fig. 2A). The same chloroplast organization was observed in plants pretreated with gamma irradiation at a dose of 20 Gy (Fig. 2B). The chloroplasts of drought-stressed plants showed an altered shape, with wavy grana and stroma thylakoids and enlarged intrathylakoidal spaces. In addition, no envelope membranes were visible in microscopic pictures of most chloroplasts (Fig. 2C). The changes observed in chloroplasts originating from drought-stressed plants pre-exposed to a low dose of gamma rays (20 Gy) were not as drastic as those observed for plants subjected only to drought stress. However, some reorganization of the thylakoids and stroma was observed (Fig. 2D).

Water deficit reduced the chlorophyll content by 12% and the photosynthetic activity by 42%. However, the chlorophyll content and the photosynthetic activity of plants irradiated with gamma rays (20 Gy) were higher than those of plants under drought-stressed conditions. Under well-watered conditions, the photosynthetic efficiency of plants irradiated with gamma rays (20 Gy) was higher than that of the control plants (Table 2). Water deficit decreased the RuBPCase activity by 27% and the PEPCase activity by 28%. However, plants irradiated with gamma rays (20 Gy) had increased RuBPCase and PEPCase activity, except for PEPCase under drought stress (Table 1). Water deficit decreased the total soluble protein concentration by 14% (Table 2).

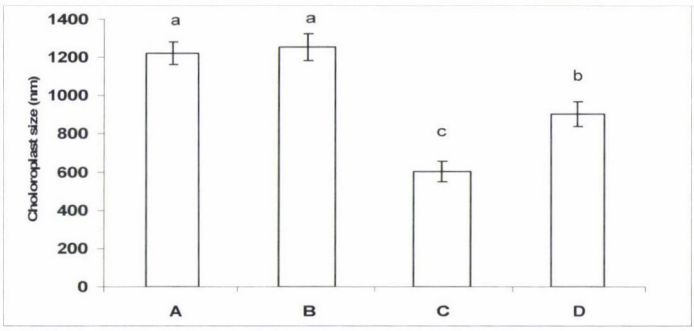

Fig. 1. Dynamic light scattering size of chloroplasts isolated from soybean leaves: (A) control; (B) plants irradiated with gamma rays (20 Gy); (C) drought-stressed plants and (D) drought-stressed plants pre-exposed to gamma irradiation (20 Gy). Values represent the means of four replicates. Different letters indicate significant differences ($P<0.05$) between treatments

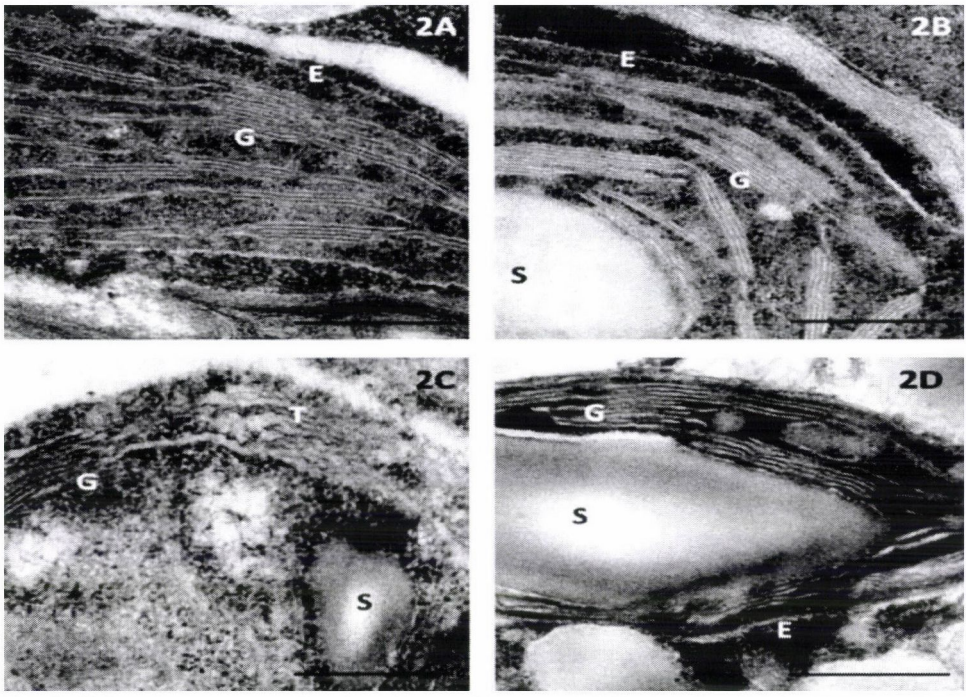

Fig. 2. Chloroplast structure of soybean leaves: (A) control; (B) plants irradiated with gamma rays (20 Gy); (C) drought-stressed plants and (D) drought-stressed plants pre-exposed to a low dose of gamma rays (20 Gy). E: envelope, G: grana, S: starch, T: thylakoid. Bars correspond to 200 nm

However, the total soluble protein content of plants pre-exposed to a low dose of gamma rays (20 Gy) was 11% higher than that of plants subjected to drought stress. Water deficit decreased the Ψ_{leaf} from –0.45 to –0.50 MPa for well-watered plants to –1.8 to –2.3 MPa for drought-stressed plants (Table 2). The application of a low dose of gamma rays (20 Gy) increased Ψ_{leaf} under drought-stressed conditions, but there was no difference in Ψ_{leaf} between irradiated plants and the control under well-watered conditions. Water deficit treatment increased the concentrations of soluble sugar, proline and MDA, the enzyme activities of POD and SOD and the electrical conductivity of the leaves (Tables 1 and 2). Under drought-stressed conditions, pre-exposure to gamma rays increased the concentrations of soluble sugars, protein and proline and the enzyme activities of POD and SOD, but not the electrical conductivity of the leaves or the concentration of MDA. For example, the application of 20 Gy gamma rays increased soluble sugar by 17% and proline by 13%, and increased SOD activity by 28% and POD activity by 30%, but the MDA concentration decreased by 13% and the electrical conductivity by 9% compared with the drought control (Tables 1 and 2).

Table 1

Effect of gamma irradiation (20 Gy) on enzyme activities of RuBPCase (μmol CO_2 min^{-1} mg^{-1} protein), PEPCase (μmol CO_2 min^{-1} mg^{-1} protein), POD (units mg^{-1} protein) and SOD (units mg^{-1} protein) and on the concentrations of MDA (nmol g^{-1} DW), proline (μmol g^{-1} DW) and total soluble protein (mg g^{-1} DW) in soybean under well-watered and drought-stressed conditions[A]

Treatments	RuBPCase	PEPCase	POD	SOD	MDA	Proline	Protein
Well-watered (W)	28.6^{b}	2.9^{b}	7.7^{c}	2.8^{c}	98^{c}	33^{c}	65^{b}
W + γ-irradiation	29.7^{a}	3.2^{a}	7.8^{c}	3.9^{b}	102^{c}	34^{c}	71^{a}
Drought-stressed (D)	20.9^{d}	2.1^{c}	11.0^{b}	4.2^{b}	130^{a}	45^{b}	56^{d}
D + γ-irradiation	23.1^{c}	2.2^{c}	14.3^{a}	5.4^{a}	115^{b}	51^{a}	62^{c}

[A] Well-watered treatment: 80% of soil field capacity; drought-stressed treatment: 35% of soil field capacity. Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests ($P<0.05$). Data are the means of four replicates.

Table 2

Effect of gamma irradiation (20 Gy) on the photosynthetic activity (*KBq mg FW^{-1}), chlorophyll content (mg g FW^{-1}), soluble sugar concentration (mg g FW^{-1}), electrical conductivity (%) and Ψ_{leaf} (MPa) of soybean under well-watered and drought-stressed conditions[A]

Treatments	Photosynthetic activity	Chlorophyll content	Soluble sugar	Electrical conductivity	Ψ_{leaf}
Well-watered (W)	16.8^{d}	52.7^{a}	117^{d}	9.6^{c}	-0.50^{a}
W + γ-irradiation	19.7^{c}	53.0^{a}	149^{c}	7.2^{d}	-0.45^{a}
Drought-stressed (D)	11.8^{b}	47.2^{c}	182^{b}	14.4^{a}	-2.3^{c}
D + γ-irradiation	14.9^{a}	49.6^{b}	213^{a}	13.2^{b}	-1.8^{b}

[A] Well-watered treatment: 80% of soil field capacity; drought-stressed treatment: 35% of soil field capacity. Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests ($P<0.05$).*kilo Becquerel (10^3 Bq). Data are the means of four replicates.

Water deficit decreased the dry weight of stems and leaves, the total biomass and the seed yield, but did not affect the dry weight of the roots (Table 3). The application of a low dose of gamma rays (20 Gy) increased the dry mass of roots, stems and leaves, and the seed yield at both water levels, with the exception of the dry weight of stems and leaves under drought-stressed conditions. Under well-watered conditions, gamma ray treatment also increased the dry weight of the roots by 55%, the stem plus leaves by 15%, the total biomass by 21% and the seed yield by 22% compared to unstressed control plants. Under drought-stressed conditions, gamma ray treatment also increased the dry weight of the roots by 22%, the stem plus leaves by 16%, the total biomass by 19% and the seed yield by 21% compared to the stressed control plants (Table 3).

Table 3
Effect of gamma irradiation (20 Gy) on the dry weight of roots and stems plus leaves, and on the seed yield and total biomass of soybean (g/plant) under well-watered and drought-stressed conditions[A]

Treatments	Roots	Stems plus leaves	Seed yield	Total biomass
Well-watered (W)	1.8^{c}	11.6^{b}	11.3^{b}	24.7^{b}
W + γ-irradiation	2.8^{a}	13.3^{a}	13.8^{a}	29.9^{a}
Drought-stressed (D)	1.8^{c}	8.1^{c}	7.9^{d}	17.8^{d}
D + γ-irradiation	2.2^{b}	9.4^{c}	9.6^{c}	21.2^{c}

[A] Well-watered treatment: 80% of soil field capacity; drought-stressed treatment: 35% of soil field capacity. Values followed by the same letter within columns are not significantly different according to Duncan's multiple range tests ($P<0.05$). Data are the means of four replicates.

Discussion

The effect of drought stress on the photosynthesis process is the subject of intensive investigation. The typical consequences of water deficit on soybean seedlings include a decrease in chloroplast size and changes in the inner chloroplast structure, registered by microscopic observations. Drought stress causes a degradation of internal chloroplast membranes, leaving the chloroplast envelopes intact. Similar findings were reported by Stoyanova et al. (2002). The changes in chloroplasts originating from drought-stressed plants pretreated with gamma irradiation (20 Gy) were not as drastic as those observed for plants subjected only to drought stress. However, some reorganization of the thylakoids and stroma was observed. These results support the findings of Wi et al. (2007). Although no conclusive explanation for the stimulatory effects of low-dose gamma radiation is yet available, it has been suggested that low-dose irradiation induces growth stimulation by changing the hormonal signalling network in plant cells or by increasing the antioxidative capacity of the cells to easily overcome daily stress factors such as fluctuations in light intensity and temperature (Kim et al., 2004). In the present study, drought-stressed soybean plants irradiated with gamma rays (20 Gy) had higher biomass and seed yield than stressed control plants. These beneficial effects resulted in higher leaf area, biomass production, grain yield and yield-related parameters in the treated plants (Moussa, 2006). Soybean plants irradiated with gamma rays (20 Gy) before the onset of water stress in the present study had improved leaf photosynthesis and chlorophyll content during the period of water stress. Abu et al. (2005) stated that an increase in chlorophyll *a*, *b* and total chlorophyll levels was observed in *Paulownia tomentosa* plants exposed to gamma irradiation. Irradiation with gamma rays (20 Gy) induced an increase in photosynthesis due to improvements in leaf water balance, as indicated by increased Ψ_{leaf} under water deficit, suggesting that the leaves lose less water. These results support the findings of previous workers (Khodary and Moussa, 2003), who reported that treating dry seeds of lupine with a low dose of gamma rays (20 Gy) increased the total

chlorophyll content, soluble sugars and photosynthetic activity. Low doses of gamma rays significantly increased the level of carbohydrate constituents (Nouri and Toofanian, 2001). SOD and POD are important antioxidant enzymes that detoxify active oxygen species. Treatment of soybean with gamma rays (20 Gy) was effective in increasing SOD and POD activity under drought stress. Similar findings were reported in *Vicia faba* by Moussa (2008), who reported that exposing three-week-old seedlings to γ-irradiation at a dose of 20 Gy increased the antioxidant enzyme activities of SOD and POD. In a study by Wi et al. (2007), it was suggested that the induction of POD by irradiation was a defence system activated by ROS-mediated cellular signalling. The enhancement of peroxidase activity by radiation was also reported by Omar (1988) in sunflower, Sah et al. (1996) in barley and Stoeva (2002) in *Phaseolus vulgaris*. The peroxidase activity in radish (*Raphanus sativus*) leaves was enhanced by gamma irradiation at 10 Gy (Lee et al., 2003). The present results also indicated that irradiating plants with gamma rays (20 Gy) promoted the accumulation of osmoprotectants, such as soluble sugars, protein and proline, and decreased the accumulation of MDA and the electrical conductivity under drought stress conditions. Osmotic electric conductivity, soluble sugars, proline and antioxidative components are used as physiological indices of membrane stability (Reddy et al., 2004). The accumulation of soluble sugars and free amino acids, including proline, protects the cell during stress by balancing the osmotic strength of the cytosol with that of the vacuole and the external environment (Kerepesi and Galiba, 2000). The results support the findings of previous workers, who stated that pre-sowing γ-irradiation at a dose of 20 Gy can be used to increase total protein content, total soluble sugars concentration, growth hormone (kinetin and GA_3), total yield and yield quality in *Eruca vesicaria* (Moussa, 2006). As a cytosolic osmoticum and a scavenger of $OH^{\cdot}$ radicals, proline can interact with cellular macromolecules such as DNA, protein and membranes and stabilize the structure and function of such macromolecules (Kavir et al., 2005). Owing to altered gene expression under gamma stress, qualitative and quantitative changes in total soluble protein content were observed (Corthals et al., 2000). These proteins might play a role in signal transduction, anti-oxidative defence, anti-freezing, heat shock, metal binding, anti-pathogenesis or osmolyte synthesis, which are essential to a plant's function and growth (Gygi et al., 1999). Ling et al. (2008) reported that a low dose of gamma irradiation (30 Gy) enhanced protein synthesis in *Citrus sinensis.*

Conclusions

This work shows for the first time that applying gamma irradiation at a dose of 20 Gy to soybean seeds prior to water deficit stress could partially alleviate the detrimental effect of water stress on growth by increasing photosynthesis, improving the antioxidant system and promoting dry weight

accumulation. Thus, it may be a useful management tool in afforestation projects in arid and semiarid areas as a promising technique for agricultural improvement.

References

Abo-Hegazi, A. M., Ragab, A. I., Moustafa, A. K. (1988): Heritability and genetic variability for some characters of sunflower in M3 and M4 generation after irradiation. *Minufia J. Agric. Res.,* **13**, 3–15.

Abu, J. O., Muller, K., Duodu, K. G., Minnar, A. (2005): Gamma irradiation of cowpea (*Vigna unguiculata* L. Walp) flours and pastes. *Food Chem.,* **95**, 138–147.

Allen, D. J., Ort, D. R. (2001): Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Sci.,* **6**, 36–42.

Barnes, J. D., Balaguer, L., Manrigue, E., Elivira, S., Davison, A. W. (1992): A reappraisal of the use of DMSO for the extraction of chlorophyll *a* and *b* in lichens and higher plants. *Environ. Exp. Bot.,* **32**, 85–90.

Bates, L. S., Waldron, R. P., Teare, I. D. (1973): Rapid determination of free proline in water stress studies. *Plant Soil,* **29**, 205–207.

Becana, M., Moran, J. F., Iturbe-Ormaetxe, I. (1998): Iron dependent oxygen free radical generation in plants subjected to environmental stresses: toxicity and antioxidants. *Plant Soil,* **201**, 137–147.

Block, A. M., Dorne, A. J., Joyard, J., Douce, R. (1983): Preparation and characterization of membrane fractions enriched in outer and inner envelope membranes from spinach chloroplasts. *J. Biol. Chem.,* **258**, 13281–13286.

Bradford, M. M. (1976): A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

Bray, G. A. (1960): A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.*, **1**, 276–285.

Chakravarty, B., Sen, S. (2001): Enhancement of regeneration potential and variability by γ-irradiation in cultured cells of *Scilla indica. Biologia Plantarum,* **44**, 189–193.

Corthals, G. L., Gygi, S. P., Aebersold, R., Patterson, S. D. (2000): Identification of proteins by mass spectrometry. *Proteome Res.,* **2**, 286–290.

Coulomb, C., Pollan, C., Lizzi, Y., Coulomb, P. J. (1996): Ultrastructural changes concerning the photosynthetic apparatus during *Phytophthora capsici* infection of susceptible and induced pepper leaves. *CR Acad Sci Paris Life Sci.,* **319**, 893–889.

Dalla Costa, L., Delle Vedove, G., Gianquinto, G., Giovanardi, R., Peressotti, A. (1997): Yield, water use efficiency and nitrogen uptake in potato: Influence of drought stress. *Potato Res.,* **40**, 19–34.

Desclaux, D., Roumet, P. (1996): Impact of drought stress on the phenology of two soybean (*Glycine max* L. Merr) cultivars. *Field Crops Res.,* **46**, 61–70.

Dhindsa, R. A., Dhindsa, P. P., Thorpe, T. A. (1981): Leaf senescence correlated with increased permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.,* **126**, 93–101.

Dornbos, D. L., Mullen, R. E., Shibles, R. M. (1989): Drought stress effects during seed fill on soybean seed germination and vigor. *Crop Sci.,* **29**, 476–480.

El-Shafie, S. A., Mazrou, M. M., El-Kholy, S. A., Sayed, S. A. (1993): Physiological influence of pre-sowing gamma irradiation on the growth, drug yield and some chemical constituents of *Ammi visnage* L. plants. *Minufia J. Agric. Res.,* **18**, 2565–2578.

Fales, F. W. (1951): The assimilation and degradation of carbohydrates by yeast cells. *J. Biology and Chemist.,* **193**, 113–124.

Gong, H., Zhu, X., Chen, K., Wang, S., Zhang, C. (2005): Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci.,* **169**, 313–321.

Gonzalez, M. C., Osuna, L., Echevarria, C., Vidal, J., Cejudo, F. J. (1998): Expression and localization of PEP carboxylase in developing and germinating wheat grains. *Plant Physiol.,* **116**, 1249–1258.

Gunes, A., Pilbeam, D. J., Inal, A., Coban, S. (2008): Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms, and lipid peroxidation. *Commun. Soil Sci. Plant Anal.,* **39**, 1885–1903.

Gygi, S. P., Rochon, Y., Franza, B. R., Aebersold, R. (1999): Correlation between protein and mRNA abundance in yeast. *Molecular Cell Biol.,* **19**, 1720–1730.

Kavir, K. P., Sangam, S., Amrutha, R. N., Sri, L. P., Naidu, K. R., Rao, K., Rao, S., Reddy, K. J., Sreenivasulu, N. (2005): Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Current Sci.,* **88**, 424–438.

Kerepesi, I., Galiba, G. (2000): Osmotic and salt stress induced alteration in carbohydrate content in wheat seedlings. *Crop Sci.*, **40**, 482–487.

Khodary, S. E., Moussa, H. R. (2003): Influence of gamma radiation and/or salinity stress on some physiological characteristics of lupine plants. *Egyptian J. Biotechnology,* **13**, 29–36.

Kim, J. H., Baek, M. H., Chung, B. Y., Wi, S. G., Kim, J. S. (2004): Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *J. Plant Biol.,* **47**, 314–321.

Korystov, Y. N., Narimanov, A. A. (1997): Low doses of ionizing radiation and hydrogen peroxidase stimulate plant growth. *Biologia (Bratislava),* **52**, 121–124.

Le Thiec, D., Manninen, S. (2003): Ozone and water deficit reduced growth of Aleppo pine seedlings. *Plant Physiol. and Biochemist.,* **41**, 55–63.

Lee, H. Y., Kim, J. S., Baek, M. H., Yoo, J. C., Kwon, S. T. (2003): Effects of low doses gamma irradiation on physiological activities of radish (*Raphanus sativus*) during early growth and reduction of gamma stress. *J. Korean Society Horticultural Sci.,* **44**, 314–320.

Ling, A. P. K., Chia., J. Y., Hussein, S., Harun, A. R. (2008): Physiological responses of *Citrus sinensis* to gamma irradiation. *World Applied Sciences Journal,* **5**, 12–19.

Liu, F. L., Jensen, C. R., Andersen, M. N. (2004): Pod set related to photosynthetic rate and endogenous ABA in soybean subject to different water regimes and exogenous ABA and BA at early reproductive stages. *Ann. Bot. (Lond),* **94**, 405–411.

Macheix, J. J., Quessada, M. P. (1984): Caractérisation d'une peroxydase impliquée spécifiquement dans la lignification, en relation avec l'incompatibilité au greffage chez l'abricotier. *Physiologie Végétale,* **22**, 533–540.

Madhava Rao, K. V., Sresty, T. V. (2000): Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. *Plant Sci.*, **157**, 113–128.

Moussa, H. R. (2006): Role of gamma irradiation in regulation of NO_3 level in rocket *(Eruca vesicaria* subsp. *sativa*) plants. *Russian Journal of Plant Physiology*, **53**, 193–197.

Moussa, H. R. (2008): Gamma irradiation effects on antioxidant enzymes and G_6PDH activities in *Vicia faba* plants. *J. New Seeds,* **9**, 89–99.

Nouri, J., Toofanian, F. (2001): Extension of storage of onions and potatoes by gamma irradiation. *Pakistan J. Biol. Sci.*, **4**, 1275–1278.

Omar, M. S. (1988): Effect of gamma ray on callus cultures and asexual embryogenesis in *Phoenix dactylifera* L. *Plant Mutation Breeding for Crop Improvement,* **6**, 258–264.

Patskevich, V. M. (1961): Conference on seed irradiation prior to sowing, *Sov. J. Atom. Energy*, **10,** 549–551.

Reddy, A. R., Chaitanya, K. V., Vivekanandan, M. (2004): Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.,* **161**, 1189–1202.

Sah, N. K., Prramanik, S., Raychaudhuri, S. S. (1996): Peroxidase changes in barley induced by ionizing and thermal radiation. *Institute J. Radiology Biol.,* **69**, 107–111.

Sánchez-Blanco, M. J., Rodríguez, P., Morales, M. A., Ortuño, M. F., Torrecillas, A. (2002): Comparative growth and water relations of *Cistus albidus* and *Cistus monspeliensis* plants during water deficit conditions and recovery. *Plant Sci.*, **162**, 107–113.

Schwertner, H. A., Biale, J. B. (1973): Lipid composition of plant mitochondria and of chloroplasts. *J. Lipid Res.,* **14**, 235–242.

Shaddad, M. A., El-Tayeb, M. A. (1990): Interactive effects of drought and phytohormones on growth and photosynthetic pigments in some crop plants. *Acta Agron. Hung.*, **39**, 1–18.

Sjodin, J. (1962): Some observations in X1 and X2 of *Vicia faba* L. after treatment with different mutagens. *Hereditas*, **48**, 565–573.

Smirnoff, N. (1993): The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist,* **125**, 27–58.

Stoeva, N. (2002): Physiological effects of the synthetic growth regulator Thidiazurol (drop) on gamma-irradiated stress in peas plants (*Pissum sativum*). *J. Central European Agric.,* **6**, 349–358.

Stoyanova, D., Tchakalova, E., Yordanov, I. (2002): Influence of different soil moisture on anatomy of maize leaves and ultrastructure of chloroplasts. *Bulg. J. Plant Physiol.,* **28**, 11–20.

Warren, C. R., Adams, M. A., Chen, Z. (2000): Is photosynthesis related to concentrations of nitrogen and Rubisco in leaves of Australian native plants? *Aust. J. Plant Physiol.,* **27**, 407–416.

Watanabe, Y., Yukawa, M., Kim, H. S., Nishimura, Y. (2000): Radiation effects on growth and seed germination of *Arabidopsis*. *Annual Report 1999–2000.* National Institute of Radiological Sciences, Anagawa, Chiba-shi, Japan.

Wi, S. G., Chung, B. Y., Kim, J. S., Kim, J. H., Baek, M. H., Lee, J. W., Kim, Y. S. (2007): Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron,* **38,** 553–564.

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WATER DEMAND AND WATER USE EFFICIENCY OF WINTER BARLEY IN HUNGARY

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The temporal and spatial variability of soil moisture, evapotranspiration and water use were investigated for winter barley. Evaluations were carried out on a database containing meteorological and yield data from 15 stations. The spatial distribution of soil moisture, evapotranspiration and water use efficiency (WUE) was evaluated from 1951 to 2000 and the moisture conditions during the growth period of winter barley were investigated. The water supply was found to be favourable, since the average values of soil moisture remained above the lower limit of favourable water content throughout the growth period, except for September–December and May–June. The actual evapotranspiration tended to be close to the potential evapotranspiration, so the water supplies were favourable throughout the vegetation period. The calculated values of WUE showed an increasing trend from 1960 to 1990, but the lower level of agricultural inputs caused a decline after 1990. The average values of WUE varied between 0.87 and 1.09 g/kg in different counties, with higher values in the northern part of the Great Hungarian Plain. The potential yield of winter barley can be calculated from the maximum value of WUE. Except in the cooler northern and western parts of the country, the potential yield of winter barley, based on the water supply, could exceed 10 t/ha.

Key words: water demand, water use efficiency, barley, soil moisture, evapotranspiration, potential yield

Introduction

Plants take up the water available in the soil. Both water and air are stored in the soil pores. This is important because the amount of water or air in the soil can only be increased at the expense of the other. Too much water decreases the air present in the pores and causes a shortage of oxygen for plants. On the other hand, too much air in the soil reduces the water content, resulting in water deficiency. Conditions are optimal if there is adequate water in the soil to facilitate plant uptake and enough air to maintain aerobic conditions.

The water in the soil must also be able to reach the assimilating organs. If the soil water is to be able to enter the roots, not only must there be a sufficient supply of water, but the ambient temperature must be above a certain threshold value. Transpiration is also necessary to ensure the flow of water from the roots to the leaves and the release of excess water into the air.

As the root system of plants is located in the soil and the stems and leaves in the air, they are equally dependent on the available water content of the soil, air humidity and evapotranspiration, which facilitates water movement from the roots to the assimilating tissues. The importance of the latter is emphasized by the fact that minerals from the soil can only reach the assimilating organs of the plants when dissolved in water, so the water supply must be continuous and uninterrupted. This is ensured by the water reserves of the soil, originating from precipitation, evapotranspiration, soil texture, and the distribution of the roots (Gregory et al., 2000; Kang et al., 2003; Zhang et al., 2004). The root density also affects the amount of water extracted from the soil (Polley, 2002). The soil water available to the crop is also influenced by tillage (López and Arrúe, 1997; Moret et al., 2007; Singh et al., 1998).

During rainy periods water input becomes dominant and the water content of the soil increases. During periods without precipitation, water loss (evapotranspiration) exceeds water input and the water content of the soil decreases.

A favoruable soil water content is essential for easy water uptake by the plants. Sufficient energy from solar radiation must also be absorbed by the plants if they are to take up water. The rate of energy and water utilization by plants is an important factor in agriculture (Ehlers and Goss, 2003). In this study the relationship between soil moisture content, evapotranspiration and the water utilization efficiency of winter barley during the growing season will be discussed.

Materials and methods

The study was based on the daily meteorological data recorded between 1951 and 2000 by the Hungarian Meteorological Service and stored in an agroclimatological data bank established by the Meteorological Team of the Institute of Mathematics, Physics and Informatics of the University of West Hungary in Mosonmagyaróvár. The database contains daily values of meteorological elements from 15 stations pertaining to this period. Using these data, potential evaporation was determined with the help of the method elaborated by Dunay et al. (1968; 1969):

$$E_0 = \frac{1-r_n}{2-r_n} \cdot t_k \tag{1}$$

where E_0 is the potential evaporation, r_n is the relative humidity of the air and t_k is the mean temperature. In order to determine the evapotranspiration of the plant canopy, it is also necessary to consider changes in the evaporating surface (leaf area) during the vegetation period. Therefore, the potential evapotranspiration (PE) can be calculated as follows:

$$PE = k(t) \cdot E_0 \tag{2}$$

where k(t) is a parameter varying over time.

The actual evapotranspiration and soil moisture were calculated with the help of a method developed by Varga-Haszonits (1991):

$$ET = \frac{PE}{1 + \exp b(\frac{w}{w_{max}})} \quad (3)$$

where ET is the actual evapotranspiration, w is the available soil water content, w_{max} is the maximum available water content in the soil and b is an empirical constant.

It was thus possible to calculate the components of the water balance from the agroclimatological point of view.

Soil moisture was expressed in terms of water availability (values between field capacity and wilting point), determined as a relative value to allow comparisons between soils with different soil moisture contents. Relative soil moisture can be calculated as follows:

$$W_r = \frac{W_a - WP}{FC - WP} = \frac{W}{W_{max}} \quad (4)$$

where W_r is the relative soil moisture, W_a is the actual soil moisture content, WP is the moisture content at the wilting point, FC is the field capacity, W is the available water and W_{max} is the maximum water availability. Plant water demand can be expressed in two forms. The moisture level required for easy water uptake from the soil is usually referred to as static water demand (Szalóky, 1989). In addition, soil moisture levels must stay above a given value to ensure a favourable rate of transpiration. This is usually called water demand for evapotranspiration or simply water demand (Posza, 1984), but can also be referred to as dynamic water demand (Szalóky, 1989).

The water use efficiency (WUE) indicates the quantity of biomass that can be produced by winter barley using a unit amount of water.

Results

An analysis was made of the soil moisture contents developing under winter barley crops during the growing season in Hungary by comparing actual values of soil moisture with the threshold value. The rate of evapotranspiration under these soil moisture conditions was then determined. Finally, the quantity of organic matter produced by the crop with unit evapotranspiration was recorded to determine the water use efficiency of winter barley in Hungary.

Soil moisture demand of winter barley

The soil water content available to plants is in the interval between field capacity and the wilting point. On heavy soils the upper limit may be just below field capacity (Szalóky, 1989). On the other hand, high water content may cause a deficit of air in the pores, causing the plants to suffer from a lack of oxygen. This is why values above 80–90% field capacity are unfavourable for plants. In the case of winter barley the lower limit of favourable soil moisture content is about 45% of field capacity (Szalóky, 1989) and the upper limit is the maximum water availability (W_{max}).

Monthly means of relative soil moisture (as a % of maximum water availability) during the growing season of winter barley are shown in Table 1. It can be seen that the mean values rarely fell below the lower limit of favourable soil moisture content (45%), and then only in September or October, so on average the soil moisture conditions during the growing season of winter barley seem to have been favourable.

Table 1
Monthly mean values of relative soil moisture content during the growing season of winter barley (1951–2000)

Location	Month									
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
Békéscsaba	0.45	0.47	0.57	0.72	0.86	0.91	0.90	0.86	0.78	0.67
Budapest	0.40	0.45	0.58	0.74	0.85	0.90	0.86	0.78	0.66	0.55
Debrecen	0.46	0.48	0.59	0.76	0.89	0.94	0.92	0.85	0.75	0.64
Győr	0.47	0.51	0.62	0.76	0.86	0.90	0.89	0.84	0.74	0.62
Iregszemcse	0.53	0.57	0.68	0.82	0.91	0.94	0.92	0.87	0.80	0.69
Kecskemét	0.42	0.46	0.60	0.78	0.90	0.94	0.90	0.81	0.70	0.57
Kompolt	0.45	0.48	0.59	0.74	0.85	0.90	0.89	0.82	0.74	0.63
Miskolc	0.52	0.54	0.63	0.75	0.84	0.89	0.88	0.83	0.77	0.68
Mosonmagyaróvár	0.53	0.57	0.68	0.82	0.91	0.94	0.94	0.90	0.81	0.68
Nyíregyháza	0.49	0.51	0.61	0.75	0.87	0.92	0.90	0.83	0.71	0.61
Pécs	0.49	0.51	0.62	0.76	0.87	0.90	0.88	0.84	0.78	0.67
Szeged	0.42	0.44	0.54	0.69	0.82	0.87	0.86	0.80	0.71	0.59
Szolnok	0.44	0.46	0.56	0.71	0.82	0.88	0.87	0.80	0.73	0.62
Szombathely	0.60	0.65	0.76	0.87	0.92	0.94	0.93	0.89	0.82	0.76
Zalaegerszeg	0.65	0.70	0.81	0.93	0.97	0.97	0.94	0.90	0.84	0.76
National mean	0.49	0.52	0.63	0.77	0.88	0.92	0.90	0.84	0.76	0.65

However, favourable mean values of soil moisture may mask more extreme values, as demonstrated by a consideration of the relative monthly minimum values of soil moisture (Table 2). It is clear from Table 2 that in some years the soil moisture level between September and January was unfavourable for winter barley. Even in January soil moisture levels were below the threshold values at two southern stations (Szeged, Pécs), because the southern part of the country is warmer and drier in this period. During the period from January to April soil moisture levels below 45% only occurred in one or two years. In June, on the other hand, despite having the highest amount of precipitation, the soil moisture content at every station is liable to drop below the threshold value. All in all, however, unfavourably low soil moisture contents are most likely to occur in the September–December period.

Water demand of winter barley for evaporation

A favourable level of soil moisture means that winter barley can take up water from the soil easily and that excess water not utilized to produce biomass evaporates into the atmosphere. The water content of the soil decreases continuously as a result of evapotranspiration during periods without replacement from precipitation or irrigation (Monteith, 1986). If the water content decreases to below the optimal soil moisture interval, water uptake becomes increasingly more difficult. It is this important to prevent soil moisture from decreasing below the critical level. This can be achieved by the usual methods of irrigation, which help to maintain a satisfactory soil moisture level.

Table 2
Monthly minimum values of relative soil moisture content during the growing season of winter barley (1951–2000)

Location	Month									
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.
Békéscsaba	0.25	0.21	0.24	0.33	0.50	0.65	0.59	0.48	0.48	0.36
Budapest	0.20	0.15	0.30	0.42	0.48	0.52	0.42	0.47	0.41	0.29
Debrecen	0.23	0.21	0.31	0.41	0.58	0.70	0.59	0.42	0.50	0.38
Győr	0.29	0.29	0.37	0.47	0.45	0.48	0.48	0.51	0.46	0.35
Iregszemcse	0.28	0.28	0.35	0.38	0.49	0.58	0.49	0.49	0.45	0.39
Kecskemét	0.19	0.19	0.25	0.32	0.58	0.65	0.50	0.48	0.38	0.29
Kompolt	0.22	0.18	0.33	0.42	0.50	0.56	0.56	0.48	0.40	0.32
Miskolc	0.25	0.21	0.31	0.36	0.54	0.60	0.59	0.45	0.48	0.35
Mosonmagyaróvár	0.32	0.38	0.44	0.56	0.55	0.58	0.61	0.64	0.56	0.36
Nyíregyháza	0.25	0.21	0.35	0.42	0.54	0.62	0.54	0.38	0.51	0.34
Pécs	0.24	0.21	0.32	0.32	0.41	0.52	0.45	0.51	0.46	0.43
Szeged	0.21	0.17	0.22	0.29	0.41	0.54	0.49	0.49	0.38	0.30
Szolnok	0.24	0.19	0.27	0.34	0.46	0.54	0.47	0.45	0.45	0.32
Szombathely	0.36	0.36	0.48	0.58	0.58	0.60	0.48	0.59	0.56	0.41
Zalaegerszeg	0.37	0.38	0.50	0.62	0.73	0.80	0.66	0.67	0.51	0.40
National mean	0.26	0.24	0.34	0.42	0.52	0.60	0.53	0.50	0.47	0.35

The question arises of how much water is necessary to compensate for water loss. According to Posza (1984) water demand (evapotranspiration demand) is defined as the quantity of water used by the crop for biomass production and evapotranspiration under the given meteorological conditions with a favourable level of soil moisture. In the latter case, water demand is equal to water uptake, which is approximately equal to the value of potential evapotranspiration (Kozmáné Tóth et al., 1995).

Water demand (WD) can thus be determined with the formula used to calculate potential evapotranspiration (PE), which is equivalent to the water input required to maintain a favourable soil moisture level:

$$WD = PE = k(t)\, E_0 \qquad (5)$$

where k(t) is a crop-specific parameter, varying over time during the growth period, and E_0 is potential evaporation, average values of which in Hungary can be seen in Table 3.

The potential evapotranspiration during the growing season of winter barley was calculated on the basis of data from the October–June period, because plants can only transpire after emerging from the soil, which usually occurs in October.

It can be seen from Table 3 that the water demand for evapotranspiration ranged from 350– 470 mm. The plants transpire to the greatest extent in spring, when soil moisture content is relatively high and the gradually increasing level of precipitation from March till June provides the plant with the necessary water quantity. Furthermore, the rise in solar radiation in spring provides sufficient energy for evapotranspiration.

Table 3
Monthly mean values of potential evapotranspiration (mm) during the growing season of winter barley (1951–2000)

Location	Month									Growing season
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	
Békéscsaba	64.3	23.3	9.1	6.5	11.6	38.7	49.0	96.7	125.3	424.3
Budapest	66.8	23.3	9.9	8.0	13.9	43.4	56.1	106.5	140.8	468.6
Debrecen	59.1	21.5	8.1	5.7	9.9	34.6	47.3	95.8	124.1	406.1
Győr	59.2	24.7	12.1	9.6	14.8	39.8	48.7	95.7	124.8	429.5
Iregszemcse	57.1	23.4	10.3	8.7	14.2	37.4	47.3	92.9	119.2	410.6
Kecskemét	60.6	22.2	9.7	7.5	12.8	37.9	49.7	101.0	136.1	437.5
Kompolt	60.9	20.0	8.0	6.7	10.1	35.8	49.2	98.0	128.8	417.6
Miskolc	50.0	16.6	6.2	5.6	8.8	33.8	46.7	91.1	119.1	377.9
Mosonmagyaróvár	49.2	20.2	10.0	8.1	12.3	32.5	42.9	85.8	111.7	372.6
Nyíregyháza	54.0	19.4	6.9	5.6	9.0	34.1	49.6	97.9	123.9	400.4
Pécs	70.7	27.1	13.2	10.3	17.4	45.2	51.0	99.6	130.2	464.6
Szeged	68.3	24.2	9.3	7.0	12.4	39.7	49.3	98.6	130.4	439.3
Szolnok	61.7	21.2	8.6	6.5	11.3	37.6	48.6	97.0	127.6	420.0
Szombathely	46.1	18.1	8.1	6.8	11.9	32.3	40.9	79.3	103.8	347.3
Zalaegerszeg	47.2	20.9	9.4	8.2	13.9	36.3	43.7	83.1	106.8	369.5
National mean	58.3	21.7	9.3	7.4	12.3	37.3	48.0	94.6	123.5	412.4

The territorial variability in water demand reflects that of solar radiation (energy for evapotranspiration) rather than that of rainfall distribution, with lower values of potential evapotranspiration (350–400 mm) in the colder western, northern and north-eastern parts of Hungary and values of 400–470 mm in the warmer, central and southern regions.

Water use efficiency of winter barley

The water use efficiency (WUE) of plants is determined from the biomass produced for each unit of water evaporated, using the formula:

$$WUE = \frac{Y}{ET} \tag{6}$$

where Y is the yield, usually expressed in kg/ha, and ET is the actual evapotranspiration during the whole growing season (usually in mm/m^2). In the present study yield was expressed in g/ha and actual evapotranspiration in kg/ha. Yield data were obtained from the Central Statistical Office, while the actual evapotranspiration data of winter barley were calculated using equation (3).

The temporal variability of actual evapotranspiration during the winter barley growing season is similar to that of potential evapotranspiration, with maximum water loss in late spring and early summer. The territorial variability is complicated by the influence of local water supplies, which can lead to regional discrepancies, as seen in the last column of Table 4. The actual evapotranspiration in Central and North-Eastern Hungary was lower than in regions with a better water supply.

Table 4
Monthly mean values of actual evapotranspiration (mm) during the growing season of winter barley (1951–2000)

Location	Month									Growing season
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	
Békéscsaba	25.7	12.1	7.1	5.9	11.0	36.2	45.1	83.5	91.1	317.8
Budapest	24.7	12.4	7.7	7.2	12.8	39.1	48.4	78.5	80.5	311.3
Debrecen	25.3	12.0	6.5	5.4	9.6	32.6	43.3	79.6	83.1	297.4
Győr	28.9	15.7	10.3	8.9	13.7	37.0	44.9	79.6	84.9	323.9
Iregszemcse	31.8	16.2	9.0	8.2	13.6	35.4	44.4	81.4	90.1	330.0
Kecskemét	23.6	12.4	8.0	7.0	12.3	35.4	43.9	75.8	77.5	296.0
Kompolt	25.8	11.5	6.5	6.0	9.4	33.3	44.2	79.7	85.1	301.5
Miskolc	26.1	11.0	5.1	5.1	8.3	31.3	42.5	77.8	88.0	295.1
Mosonmagyaróvár	28.7	14.7	8.9	7.7	11.8	31.3	41.0	76.9	85.5	306.6
Nyíregyháza	25.6	11.4	5.6	5.2	8.6	31.7	45.0	77.9	78.1	289.1
Pécs	34.7	16.7	10.6	9.4	16.2	41.7	46.6	84.6	93.9	354.4
Szeged	25.1	11.8	6.9	6.2	11.4	35.7	42.8	75.8	80.3	296.1
Szolnok	23.9	11.3	6.4	5.6	10.3	34.3	43.1	78.1	84.0	297.1
Szombathely	32.4	14.8	7.5	6.5	11.3	30.5	39.0	71.4	86.1	299.6
Zalaegerszeg	36.0	17.9	9.0	8.0	13.6	35.1	41.8	75.3	88.3	324.9
National mean	27.9	13.5	7.7	6.8	11.6	34.7	43.7	78.4	85.1	309.4

The WUE data in Table 5 show that winter barley had a mean grain yield of 0.87–1.09 g for every kg of water evaporated, with maximum values of 1.41–2.14 g/kg. Higher values were found in the northern part of the Great Hungarian Plain, in Jász-Nagykun-Szolnok, Hajdú-Bihar and Szabolcs-Szatmár-Bereg counties. The minimum values of WUE ranged from 0.29–0.51 g/kg. These results are consistent with the range evaluated by other authors (López and Arrúe, 1997; Moret et al., 2007; Gregory et al., 1992; Cantero-Martinez et al., 1996).

The WUE of winter barley is also influenced by the variety and production technology. As seen in Figure 1, the use of newer, intensive varieties and the application of improved technology led to continuously increasing values of WUE for winter barley from 1960–1990, after which the decline in agricultural inputs caused a decrease in water use efficiency.

WUE-based climatic potential of winter barley

The maximum potential yield (Y_{max}), also called the WUE-based climatic potential, can be calculated from WUE values by rewriting equation (6) in the form:

$$Y = WUE.ET \qquad (7)$$

and substituting the maximum values of WUE for a given territory (WUE_{max}) and of potential evapotranspiration (PE_{max}) in place of actual evapotranspiration (ET), as follows:

$$Y_{max} = WUE_{max}.PE_{max} \qquad (8)$$

Table 5
Values of WUE for winter barley (1951–2000)

Location	Water use efficiency(g/kg)		
	Maximum	Mean	Minimum
Békéscsaba	1.79	1.03	0.37
Budapest	1.86	0.91	0.51
Debrecen	2.10	0.99	0.41
Győr	1.85	1.02	0.40
Iregszemcse	1.93	1.09	0.32
Kecskemét	1.82	0.99	0.43
Kompolt	1.72	0.95	0.42
Miskolc	1.89	0.87	0.41
Mosonmagyaróvár	1.81	1.07	0.43
Nyíregyháza	2.14	0.91	0.51
Pécs	1.82	0.95	0.29
Szeged	1.56	0.97	0.42
Szolnok	2.06	1.05	0.41
Szombathely	1.67	0.94	0.40
Zalaegerszeg	1.41	0.87	0.44

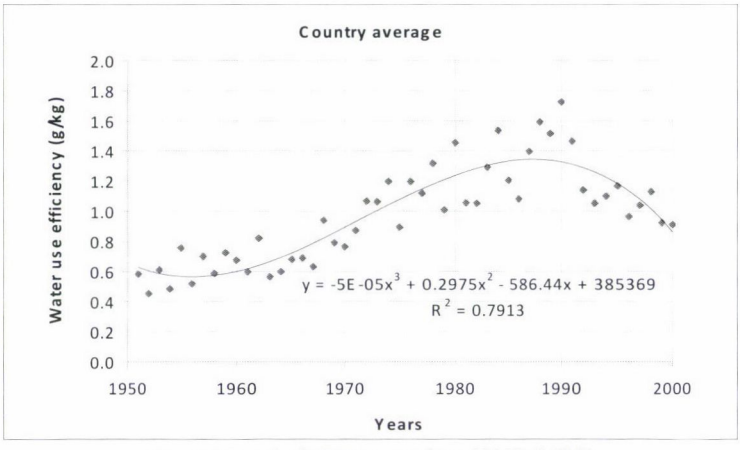

Fig. 1. Trend of WUE over time (1951–2000)

If the potential yield is to be expressed in kg/ha, WUE_{max} must be converted to kg/kg and PE_{max} to kg/ha.

Maximum values of WUE and potential evapotranspiration at different locations during the growing season of winter barley in the second half of the 20th century are shown in the third column of Table 6. If these maximum values had occurred in the same year at the same location and the values of all other factors had been optimal, the potential yields shown in the last column would have been produced. It is clear from the table that the maximum potential yield is less than 10 t/ha in the cooler northern and western counties, and above 10 t/ha elsewhere, with the exception of Békéscsaba, in the south-eastern part of the Great Hungarian Plain.

Table 6
WUE based potential yield of winter barley (1951-2000)

Location	WUE_{max} (g/kg)	PE_{max} (kg/m^2)	Y_{max} (kg/ha)
Békéscsaba	1.79	507.4	9034
Budapest	1.86	606.4	11279
Debrecen	2.10	486.9	10225
Győr	1.85	547.8	10134
Iregszemcse	1.93	540.2	10426
Kecskemét	1.82	572.1	10412
Kompolt	1.72	530.1	9118
Miskolc	1.89	498.6	9424
Mosonmagyaróvár	1.81	524.9	9501
Nyíregyháza	2.14	527.1	11280
Pécs	1.82	617.9	11246
Szeged	1.56	566.2	8833
Szolnok	2.06	571.3	11769
Szombathely	1.67	504.7	8429
Zalaegerszeg	1.41	515.7	7271

Conclusions

Yields are influenced by technological factors (crop varieties, nutrient supply, plant protection) and meteorological factors. If the technological factors are favourable (high-yielding varieties, optimal nutrient supply, satisfactory control of weeds, diseases or pests), water conditions have a greater effect on productivity than temperature, due to its greater variability. The water supply during the growth period of winter barley was thus investigated in terms of soil moisture content and evapotranspiration. In Hungary the vegetation period of winter barley is cool and wet and the water supply is relatively favourable.

In some years, however, the soil moisture level may be below the lower threshold value from September to December and again in May and June.

The water use efficiency of winter barley is influenced not only by the water supply, but also by the supply of nutrients. The water use of crops changes parallel with the standard of the technology, as indicated in Figure 1. Water use efficiency increased during the 1960–1990 period, but decreased after 1990 due to changes in the technology. In order to optimize WUE, decisions on cultivar and nutrient management must be coordinated. Water use efficiency can be increased through proper management, and these changes have a positive effect on crop yields (Hatfield et al., 2001).

Provided the water supply, nutrient supply and plant protection are favourable, the WUE-based potential yields in cooler northern and western counties will be below 10 t/ha, while the maximum values attainable in all other territories are potentially above 10 t/ha under the climatic conditions of Hungary.

References

Cantero-Martinez, C., Villar, J. M., Romagosa, I., Fereres, E. (1996): Growth and yield responses of two contrasting barley cultivars in a Mediterranean environment. *Eur. J. Agron.*, **4**, 317–326.

Dunay, S., Posza, I., Varga-Haszonits, Z. (1968): Egyszerű módszer a tényleges evapotranszspiráció és a talaj vízkészletének meghatározására. I. A párolgás meteorológiája. (A simple method for determining actual evapotranspiration and soil moisture content. I. Meteorology of evaporation). *Öntözéses Gazdálkodás*, **6**(2), 39–48.

Dunay, S., Posza, I., Varga-Haszonits, Z. (1969): Egyszerű módszer a tényleges evapotranszspiráció és a talaj vízkészletének meghatározására II. (A simple method for determining actual evapotranspiration and soil moisture content. II.) *Öntözéses Gazdálkodás*, **7**(2), 27–38.

Ehlers, W., Goss, M. (2003): *Water Dynamics in Plant Production.* CABI Publishing. Wallingford, UK.

Gregory, P. J., Simmonds, L. P., Pilbeam, C. J. (2000): Soil type, climatic regime, and the response of water use efficiency to crop management. *Agron. J.*, **92**, 814–820.

Gregory, P. J., Tennant, D., Belford, R. K. (1992): Root and shoot growth, and water and light use efficiency of barley and wheat crops grown on a shallow duplex soil in a Mediterranean-type environment. *Aust. J. Agric. Res.*, **43**, 555–573.

Hatfield, J. L., Sauer, T. J., Prueger, J. H. (2001): Managing soils to achieve greater water use efficiency. *Agron. J.*, **93**, 271–280.

Kang, S., Zhang, L., Liang, Y., Dawes, W. (2003): Simulation of winter wheat yield and water use efficiency in the Loess Plateau of China using WAVES. *Agric. Systems*, **78**, 355–367.

Kozmáné Tóth, E., Posza, I., Tiringer, C. (1995): Szántóföldi növényállományok vízigénye, tényleges párolgása és öntözővízszükséglete. (Water demand, actual evaporation and irrigation demand of plant stands in the field.) *Éghajlati és Agrometeorológiai Tanulmányok*, **3**, 33–96.

López, M. V., Arrúe, J. L. (1997): Growth, yield and water use efficiency of winter barley in response to conservation tillage in a semi-arid region of Spain. *Soil Till. Res.*, **44**, 35–54.

Monteith, J. L. (1986): How do crops manipulate water supply and demand? *Philos. Trans. R. Soc. Ser. A.*, **316**, 245–259.

Moret, D., Arrúe, J. L., López, M. V., Gracia, R. (2007): Winter barley performance under different cropping and tillage systems in semiarid Aragon (NE Spain). *Eur. J. Agron.*, **26**, 54–63.

Polley, H. W. (2002): Implication of atmospheric and climatic change for crop yield and water use efficiency. *Crop Sci.*, **42**, 131–140.

Posza, I. (1984): *Növényállományok evapotranszspirációja.* (Evapotranspiration of plant stands.) PhD dissertation. Szarvas. 111 p.

Singh, B., Chanasyk, D. S., McGill, W. B. (1998): Soil water regime under barley with long-term tillage-residue systems. *Soil Till. Res.*, **45**, 59–74.

Szalóky, S. (1989): A növények vízigénye, vízhasznosítása és öntözővíz-szükséglete. pp. 100–154. In: *Water demand, water use and irrigation demand of crops. (Practical manual of irrigation.)* Szalai, G. (ed.), Az öntözés gyakorlati kézikönyve. Mezőgazdasági Kiadó, Budapest.

Varga-Haszonits, Z. (1991): *Az őszi búza vízellátottsága és vízfogyasztása.* (Water supply and water use of winter wheat.) OMSz, Budapest, pp. 103–117.

Zhang, Y. Q., Kendy, E., Yu, Q. A., Liu, C. M., Shen, Y. J., Sun, H. Y. (2004): Effect of soil water deficit on evapotranspiration, crop yield, and water use efficiency in the North China Plain. *Agric. Water Manag.*, **64**, 107–122.

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REGIONAL ANALYSIS OF WINTER WHEAT YIELDS UNDER DIFFERENT ECOLOGICAL CONDITIONS IN HUNGARY AND CROATIA

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Wheat is the second most important field crop on arable lands in Hungary and Croatia. Yield variations between years are high in both countries. In the short term these variations are mainly the result of the weather parameters specific to individual growing seasons. The aim of this study was to compare variations in winter wheat yields over years in four counties in Hungary and five in Croatia, with the emphasis on the impact of rainfall and mean air temperature regimes. The results showed that rainfall in spring was most decisive for winter wheat yields. The highest winter wheat yields were obtained when the rainfall in the winter half-year ranged from 230–260 mm and the spring rainfall from 180–230 mm. The precipitation in the growing season is much higher in eastern Croatia than in eastern Hungary, so water shortage is a more pronounced environmental problem for wheat in Hungary. This is probably why wheat yields were lower in eastern Hungary than in eastern Croatia in the period tested. Pearson correlation analysis on the yields and meteorological data between 1990 and 2009 revealed a positive correlation between spring rainfall and the yield, and a negative correlation between spring temperature and the yield. The results proved that yields were determined not only by weather conditions, but by many other factors (crop rotation, tillage, fertilization, variety, crop protection, etc.).

Key words: winter wheat, yield, ecological conditions

Introduction

Wheat is the second most important field crop on arable lands in Hungary and Croatia. Between 2005 and 2007 wheat was grown on 1 106 000 ha year^{-1} in Hungary and on 165 721 ha year^{-1} in Croatia, representing about 24.5% and 20.0% of the total arable land in Hungary and Croatia, respectively.

Yield variations over years are high in both countries, with yields ranging from 2640 to 5120 kg ha^{-1} in Hungary and from 2960 to 4640 kg ha^{-1} in Croatia between 1998 and 2007. In the short term these variations are mainly the result of weather parameters specific to individual growing seasons. In general, low

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wheat yields in Eastern Croatia are mainly due to excess rainfall, especially during the autumn/winter period, or to drought stress (Josipovic et al., 2005; Kovacevic, 2005; Kovacevic and Josipovic, 1995; Kovacevic et al., 2009; Marijanovic et al., 2010). In Hungary drought stress is the main factor limiting the yield of wheat (Pepó, 2009a).

Over the last 20 years there have been many economic and agronomic changes in Hungarian wheat production. For financial reasons the level of inputs (fertilizers, pesticides, etc.) has dropped. There have also been changes in the agroecological conditions. As the result of global climate change crop yields have declined and yield fluctuation has increased, as reported by Olsen and Bindi (2002), Birkás et al. (2006), Várallyay (2007), Balogh and Pepó (2008), Pepó (2009b) and Vad and Dóka (2009). The unfavourable effects of abiotic stress can be reduced by a wise choice of variety and the optimum use of production technology. Among the agronomic factors, optimum nutrient and water supplies are outstandingly important (Jolánkai, 1982; Ruzsányi, 1991; Berzsenyi, 1993; Pepó, 2004a; 2009b; Vad and Dóka, 2009), but other factors may modify their effects.

Yield losses in winter wheat in different years depended on the soil conditions, the stress tolerance of the variety and the technology. These losses varied from 2–55% (Baginskas et al., 1985; Zhao, 1987; Kosminski et al., 1994; Shen et al., 1999; Domitruk et al., 2000; Pepó, 2004b).

Pepó (2009c) proved that the effects of agroecological conditions could be modified by using different crop models. In the case of non-intensive wheat production the ecological conditions (soil, weather) had a 60% effect on the wheat yield, whereas the use of intensive technology reduced this figure to 25%. The aim of this study was to compare winter wheat yield variations over years in the eastern parts of Hungary and Croatia, with the emphasis on the impact of rainfall and mean air temperature regimes.

Materials and methods

Data collection

Official data on grain yields, rainfall and mean air temperatures in Budapest (Hungary) and Zagreb (Croatia) were used in this study for the periods 1990–2009 (Hungary) and 1990–2007 (Croatia). It should be noted that the data for Croatia are incomplete for 1991–1995 due to the war, while the 2008 and 2009 Statistical Yearbooks for Croatia only contain field crop yield data at national level. Four counties in eastern Hungary and five in eastern Croatia were chosen for the study, and weather parameters were collected from four meteorological stations in each country.

Rainfall and air temperature parameters were collected for the growing season (October to June), the spring period (March to June) and the winter half-year (September to February), for each month separately and averaged over the seasons (Tables 2 and 5).

The data were provided by our meteorological stations in each country. In Hungary: Debrecen (47°32' N, 21°38' E; elevation above sea level = 120 m), Szolnok 47°11' N, 20°11' E; 85 m), Nyíregyháza (48°08' N, 21°28' E; 115 m) and Békéscsaba (46°41' N, 21°05' E; 87 m); in Croatia: Gradiste, near Zupanja (45° 09' N, 18°43'E; 87 m), Osijek (45° 33' N, 18°41' E; 94 m), Slavonski Brod (45° 09' N, 18°01' E; 93 m) and Bjelovar (45° 54', 16°51' E; 133 m).

Pearson correlation analysis was performed on the winter wheat yields and meteorological data.

Description of tested areas

Four counties in the Trans-Tisza region of eastern Hungary were involved in the experiments, namely Hajdú-Bihar county (HB), Jász-Nagykun-Szolnok county (JNS), Szabolcs-Szatmár-Bereg county (SSB) and Békés county (B), which together covered 25.2% of the territory of Hungary (6.7% + 6.0% + 6.4% + 6.1%, respectively), on which 32.4% of the national wheat yield was harvested in 2009 (12.3% HB, 23.5% JNS, 5.7% SSB, 22.1% B). The soil conditions are more favourable in Békés and Hajdú-Bihar than in Szabolcs-Szatmár-Bereg and Jász-Nagykun-Szolnok, which have mainly sandy and salty loamy soils, respectively. The agrometeorological conditions also varied in the different counties.

The region studied in Eastern Croatia covered 12 454 km^2 or about 22% of the country. In general, soil fertility declined from the east to the west part of the region (Janekovic, 1971; Škorić, 1977), with eutric cambisols, gleysols and calcaric fluvisol dominating in the east and fertile soils (luvisols, stagnosols and planosols) in the west, leading to differences in crop yields. The five counties studied were Vukovar-Sirmium (VS: 2454 km^2), Osijek-Baranya (OB: 4155 km^2), Brod-Posavina (BP: 2030 km^2), Pozega-Slavonia (PS: 1823 km^2) and Virovitica-Podravina (VP: 2024 km^2).

Eastern Croatia has a moderate continental climate characterized by low horizontal changes in air temperature and more rainfall in the warmer part of the year (early April to late September), though droughts are possible in July and August. The mean monthly air temperature is about 22°C.

Results and discussion

Between 1990 and 2009 the average wheat yield was the highest in county B (4261 kg ha^{-1}), but only slightly lower in county HB (4247 kg ha^{-1}). The maximum yields in SSB and JNS were 3710 kg ha^{-1} and 3623 kg ha^{-1}. The highest maximum yield was recorded in county B (6190 kg ha^{-1} in 2004), while the maximum yields in the other counties were 5770 kg ha^{-1} in HB and in 5270 kg ha^{-1} in SSB (both in 1991). The minimum yields were recorded in very dry years, with values of 2890 kg ha^{-1} in county B (1993), 3050 ha^{-1} in county HB, 2550 kg ha^{-1} in SSB and 1890 kg ha^{-1} in JNS (2003) (Table 1).

An evaluation was made of the effect of meteorological parameters (rainfall in the winter half-year, spring period and vegetation period and average temperature in spring and the vegetation period) on the yields of winter wheat (Table 2). Between 1990 and 2009 there were substantial fluctuations in rainfall quantities in the winter half-year (105–373 mm), in spring (46–363 mm) and in the vegetation period (236–629 mm), while the average temperature in spring (11.8–15.6°C) and during the vegetation period (6.2–10.9°C) varied to a lesser extent. The results indicate that spring rainfall had the greatest effect on the yield of winter wheat. If the rainfall was 230–260 mm in the winter half-year and 180–230 mm in spring this resulted in the highest winter wheat yields in the counties investigated (in 1991 and 2004). Different results were obtained in county SSB because of the special soil conditions. The lowest yields were recorded if the rainfall in spring was extremely low. The minimum yields varied between 1890 (JNS) and 3050 kg ha^{-1} (HB). In these cases the rainfall in the winter half-year was normal (210–260 mm), but the precipitation in spring was very low (46–120 mm) and there were high temperatures in the spring months. In the dry years (1993 and 2003) very poor wheat yields were recorded (1890–3050 kg ha^{-1}) in all the counties.

Table 1
Variation in winter wheat yields (kg ha^{-1}) and fluctuation range (%) in four counties of Eastern Hungary[+]

County	1990–2009				1990–1999				2000–2009			
	Max.	Min.	Mean	Range	Max.	Min.	Mean	Range	Max.	Min.	Mean	Range
HB	5770	3050	4247	72–136	5770	3120	4331	72–133	5500	3050	4163	73–132
JNS	5240	1890	3623	52–145	5240	2370	3784	63–138	5180	1890	3460	55–150
SSB	5270	2550	3710	69–142	5270	2690	3748	72–141	4530	2550	3699	69–122
B	6190	2890	4261	68–145	5770	2890	4329	67–133	6190	2970	4173	71–148

Hajdú-Bihar (HB), Jász-Nagykun-Szolnok (JNS), Szabolcs-Szatmár-Bereg (SSB) and Békés (B);

The precipitation in the growing season ranged from 251–534 mm over the 1990–2009 period in Debrecen. In general, lower wheat yields were recorded in dry years. The five growing seasons with less than 300 mm precipitation (mean 273 mm) had a mean wheat yield of 3949 kg ha^{-1}, while the five with more than 400 mm precipitation (mean 455 mm) had a mean yield of 4542 kg ha^{-1} (Hajdú-Bihar county). The mean air temperatures in these growing seasons were 7.9°C in the dry years and 7.0°C in the wet years (Table 2). A similar relationship was found between spring precipitation and yields of wheat. Low spring precipitation (less than 160 mm) in six growing seasons (mean 144 mm) was associated with a mean yield of 4070 kg ha^{-1}, while with higher spring precipitation (eight years above 200 mm) the mean yield was 4603 kg ha^{-1}, i.e. 13% higher. The mean air temperatures were 13.5 and 13.0°C in the dry and wet springs, respectively (Table 2).

The precipitation in the growing season of wheat was considerably higher in eastern Croatia than in eastern Hungary (average 1961–1990: 482 mm in Osijek and 361 mm in Debrecen: Tables 2 and 3). Based on the data of the Osijek and Debrecen meteorological stations, only two years in Osijek and five in Debrecen had less than 300 mm precipitation between 1990 and 2009 (dry growing seasons), while 9 years in Osijek and 1 in Debrecen were wet growing seasons (above 500 mm).

The distribution of spring precipitation over this period also differed for the two regions, with less than 160 mm rainfall in two years in Osijek and six in Debrecen, and over 200 mm precipitation in 13 years in Osijek and 8 in Debrecen, suggesting that water shortage is a more pronounced environmental problem for wheat production in eastern Hungary. This probably accounts for the lower wheat yields in eastern Hungary (Hajdú-Bihar county: 4045 kg ha^{-1}) in comparison with eastern Croatia (Osijek-Baranya county: 4576 kg ha^{-1}) in the tested period (Tables 2 and 3).

Table 2
Meteorological data and wheat yields for four counties in Eastern Hungary[+]

Year	Debrecen (HB)			Szolnok (JNS)			Nyíregyháza (SSB)			Békéscsaba (B)		
	Spr.	Gr.S	Yield	Spr.	Gr.S	Yield	Spr.	Gr.S	Yield	Spr.	Gr.S	Yield
Rainfall (mm) and yields of wheat (kg ha^{-1})												
1990	136	264	5480	138	236	4460	131	219	5270	195	334	5670
1991	177	387	5770	196	382	5240	209	391	4700	224	398	5770
1992	122	348	4130	153	330	3520	145	339	3570	118	356	3940
1993	188	388	3120	102	266	2370	148	327	2690	117	301	2890
1994	202	453	5300	178	380	4780	152	328	4260	183	402	5080
1995	215	365	4180	208	348	3440	213	367	3850	289	449	4140
1996	169	320	3710	184	381	3310	111	220	2770	200	446	3540
1997	163	278	4150	159	284	3790	168	331	3780	210	378	4350
1998	234	336	3900	197	339	3800	288	433	3010	244	406	4420
1999	232	464	3560	248	476	3130	181	441	3580	256	512	3490
2000	186	381	3840	137	327	2910	143	337	3300	144	453	3720
2001	256	396	4730	216	361	4040	202	353	4370	357	477	4490
2002	162	251	3680	169	254	2420	152	230	3380	123	234	3390
2003	118	271	3050	46	235	1890	120	301	2550	78	266	2970
2004	199	407	5500	213	409	5180	191	419	4530	232	445	6190
2005	245	419	4490	190	367	4150	204	394	4380	175	401	4510
2006	363	534	3860	276	431	3030	336	518	3960	238	408	3730
2007	202	301	3386	136	264	3092	131	250	3348	211	333	3860
2008	245	393	5167	222	379	4689	175	343	4510	331	480	5000
2009	162	328	3930	174	349	3200	195	346	2660	123	332	4070
Average air temperature (°C) and yields of wheat (kg ha^{-1})												
1990	13.4	8.0	5480	14.0	8.5	4460	13.1	7.6	5270	13.8	8.3	5670
1991	12.1	6.8	5770	12.2	6.8	5240	11.9	6.4	4700	12.3	7.0	5770
1992	13.2	6.9	4130	13.7	7.6	3520	12.7	6.5	3570	13.5	7.3	3940
1993	12.8	6.7	3120	13.5	7.4	2370	12.6	6.5	2690	13.3	7.1	2890
1994	13.4	8.2	5300	14.0	8.4	4780	13.3	7.9	4260	13.8	8.7	5080
1995	12.4	7.4	4180	13.0	8.1	3440	12.2	7.1	3850	12.3	7.6	4140
1996	12.6	6.5	3710	12.9	6.5	3310	12.7	6.2	2770	12.5	6.5	3540
1997	11.8	6.8	4150	12.3	7.2	3790	11.8	6.7	3780	12.0	7.1	4350
1998	13.1	8.5	3900	13.0	8.4	3800	12.7	7.8	3010	12.7	8.2	4420
1999	13.6	6.6	3560	14.0	7.1	3130	13.8	6.7	3580	14.0	7.2	3490
2000	14.4	7.8	3840	15.0	8.4	2910	14.5	7.8	3300	14.6	8.2	3720
2001	13.4	8.9	4730	13.7	9.2	4040	13.3	8.8	4370	13.9	9.5	4490
2002	14.5	7.8	3680	15.0	8.5	2420	14.5	7.9	3380	14.9	8.3	3390
2003	13.8	6.7	3050	14.6	7.2	1890	13.6	6.6	2550	14.3	7.3	2970
2004	12.6	6.8	5500	13.2	7.7	5180	12.0	6.4	4530	13.3	7.7	6190
2005	11.9	6.7	4490	13.5	8.6	4150	12.0	6.6	4380	12.7	7.0	4510
2006	12.3	6.6	3860	13.4	7.6	3030	12.1	6.1	3960	12.9	7.1	3730
2007	15.6	10.0	3386	16.1	10.9	3092	15.1	9.7	3348	15.6	10.3	3860
2008	13.8	8.0	5167	14.6	8.7	4689	13.4	7.7	4510	14.4	8.6	5000
2009	14.4	8.7	3930	15.1	9.3	3200	13.7	8.2	2660	14.7	9.0	4070
30-year mean (1961–1990)												
mm	215	406	–	194	361	–	185	346	–	219	417	–
°C	12.6	6.9	–	12.9	7.3	–	12.6	6.9	–	12.9	7.4	–

[+]Rainfall and average air temperatures in spring (Spr.=March–June) and the growing season (Gr.S = Oct.–June) and wheat yields at county level: Hajdú-Bihar (HB), Jász-Nagykun-Szolnok (JNS), Szabolcs-Szatmár-Bereg (SSB) and Békés (B)

Table 3
Meteorological data and grain yields of wheat for four counties in Eastern Croatia[+]

Year	Gradiste (VS)			Osijek (OB)			Slavonski Brod (BP)			Bjelovar (VP)		
	Spr.	Gr.S	Yield	Spr.	Gr.S	Yield	Spr.	Gr.S	Yield	Spr.	Gr.S	Yield
Rainfall (mm) and yields of wheat (kg ha^{-1})												
1990	165	311	6720	192	351	6770	179	326	5680	243	379	6040
1991	245	455	5770	245	449	6040	280	526	5020	222	418	4400
1992	186	469	4880	224	–	4960	249	537	4310	200	437	4310
1993	172	526	5040	221	564	5210	181	628	4300	165	522	4730
1994	269	598	4740	210	517	4600	297	685	3780	254	733	3830
1995	284	553	4490	272	541	4500	285	546	3850	327	614	4330
1996	280	501	4260	232	478	4240	264	504	3890	247	535	3990
1997	152	489	4730	206	520	4830	243	553	4160	212	531	4100
1998	221	580	4660	150	476	5060	206	437	4460	318	570	4620
1999	294	500	3720	313	606	3690	265	525	3340	359	740	3300
2000	181	468	5130	105	382	5120	149	420	4750	196	419	4680
2001	437	637	4790	455	642	5020	375	609	4090	302	573	4520
2002	218	471	4970	246	418	4870	262	477	4050	255	435	4560
2003	91	468	3500	79	284	3280	136	324	3130	153	436	3210
2004	402	733	–	319	623	–	366	752	–	352	646	–
2005	211	536	4630	247	600	4290	286	608	4170	288	622	3850
2006	289	450	4840	310	524	5010	315	531	4060	268	463	4490
2007	276	410	5140	168	264	4870	239	434	4760	262	402	4250
2008	263	565		278	560		326	630		284	531	
2009	179	421		148	356		202	447		218	533	
Average air temperature (°C) and yields of wheat (kg ha^{-1})												
1990	14.4	9.5	6720	14.5	9.2	6770	14.0	9.9	5680	13.8	9.2	6040
1991	12.9	8.0	5770	12.8	7.7	6040	12.7	7.7	5020	12.4	7.1	4400
1992	14.1	8.6	4880	13.9	–	4960	13.9	8.4	4310	13.7	8.2	4310
1993	14.1	8.5	5040	13.9	8.6	5210	14.2	8.6	4300	13.4	7.8	4730
1994	14.9	9.2	4740	14.6	8.8	4600	14.7	8.9	3780	14.1	8.4	3830
1995	13.1	8.9	4490	12.8	8.5	4500	12.7	8.7	3850	12.8	8.3	4330
1996	13.6	7.7	4260	13.4	7.4	4240	13.3	7.7	3890	12.9	7.2	3990
1997	13.3	8.6	4730	13.1	8.2	4830	13.0	8.4	4160	13.2	8.1	4100
1998	14.0	9.4	4660	13.7	9.0	5060	13.7	8.8	4460	13.7	8.9	4620
1999	14.9	8.5	3720	14.6	8.1	3690	14.6	8.0	3340	15.0	8.2	3300
2000	15.8	9.4	5130	15.7	9.1	5120	15.0	6.2	4750	15.9	9.4	4680
2001	14.7	10.6	4790	14.3	10.1	5020	14.3	9.8	4090	14.7	10.4	4520
2002	15.4	9.6	4970	14.8	8.7	4870	14.8	8.6	4050	15.7	9.3	4560
2003	15.9	9.1	3500	15.4	8.6	3280	15.1	8.8	3130	16.3	9.5	3210
2004	13.5	8.5	–	12.8	7.8	–	13.1	8.3	–	13.3	8.4	–
2005	13.4	8.3	4630	13.0	7.8	4290	13.1	7.1	4170	13.2	8.1	3850
2006	13.9	8.4	4840	13.6	8.0	5010	13.5	7.9	4060	13.5	8.0	4490
2007	16.0	11.4	5140	15.6	10.9	4870	15.7	10.9	4760	15.7	11.2	4250
2008	15.4	9.4		14.9	8.9		14.8	8.9		14.7	8.9	
2009	15.2	9.7		14.7	9.4		14.7	9.3		14.8	9.4	
Long-term mean												
	1971–1990			1961–1990			1961–1990			1961–1990		
mm	242	486	–	245	482	–	267	532	–	291	589	–
°C	13.7	8.4	–	13.3	7.9	–	13.0	7.7	–	12.8	8.0	–

[+]Rainfall and average air temperatures in spring (Spr. = March–June) and the growing season (Gr.S: Oct.–June) and wheat yields at county level: Vukovar-Sirmium (VS), Osijek-Baranya (OB), Brod-Posavina (BP) and Virovitica-Podravina (VP)

The monthly precipitation and temperature regimes (Tables 4 and 5) could be a better criterion for characterizing the favourability of individual growing seasons with respect to wheat production. The 1989/1990 growing season was favourable for wheat in Croatia with moderate, well-distributed precipitation and air temperatures in Osijek that were 1.3°C higher than the long-term mean. The winter was especially mild (January + February = 5.4°C; mean 2.2°C). The next growing season was also favourable for wheat, having precipitation and temperature values on par with the long-term means. The coldest month was February, after which the temperature slowly increased (being somewhat colder in March, April and May compared to the long-term mean). Two less favourable growing seasons in Croatia were 1998/1999 and 2004/2005, with higher precipitation with two peaks (in two autumn months and in June). The very cold weather in December (–3.3°C) and the higher spring temperatures (April–June 16.7°C) were additional unfavourable factors in the 1998/1999 growing season. The main characteristics of the less favourable growing season of 2002/2003 in Croatia were drought (Osijek: 61 mm precipitation in the four-month period February–May), the very cold winter (January and February: –2.7°C) and the high temperatures in May and June (mean 22.2°C). In Debrecen, the precipitation sums and air temperatures during the growing season of wheat are usually lower than in Osijek (long-term means 420 mm and 6.9°C compared to 482 mm and 7.9°C). The winters are somewhat colder in Debrecen (January and February: –1.4°C compared to 0.2°C). The factors making the growing season less favourable in the Trans-Tisza region are mainly drought stress and cold winters (Tables 4 and 5).

The results of Pearson correlation analysis on the yields and meteorological data of four counties in the period 1990–2009 (Table 6) revealed a positive correlation between spring rainfall and wheat yields and a negative correlation between spring temperature and wheat yields. A particularly strong correlation was noted for county JNS, with values of $R^2 = 0.451$ for rainfall and $R^2 = -0.424$ for temperature (Table 6). Very loose correlations were detected for the other counties (HB, SSB and B), suggesting that wheat yields in these counties were determined not only by weather conditions, but by many other factors (crop rotation, tillage, fertilization, variety, crop protection, etc.).

Conclusions

In the short-term yield variations over years in Hungary and Croatia are mainly the result of the weather parameters specific to individual growing seasons. Data for the 1990–2009 period showed that the rainfall in spring had the most decisive influence on the yields of winter wheat. Precipitation sums in the growing season of wheat are considerably higher in eastern Croatia than in eastern Hungary (average 1961–1990: 482 mm in Osijek and 361 mm in Debrecen). A positive correlation was detected between the rainfall in spring and the wheat yield and a negative correlation between the temperature in spring and the yield of winter wheat.

Table 4
Precipitation and mean air temperatures (data from the Debrecen meteorological station) and wheat grain yields in the Trans-Tisza region of eastern Hungary

Year	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Σ	Yield kg ha^{-1}
	Precipitation (mm)										
1990	21	46	14	23	24	6	52	28	50	264	5480
1991	57	44	53	12	44	15	47	93	22	387	5770
1992	104	45	48	16	13	7	28	40	47	348	4130
1993	95	55	18	14	18	38	60	19	71	388	3120
1994	38	81	59	30	43	21	91	59	31	453	5300
1995	21	11	33	34	51	26	47	46	96	365	4180
1996	0	49	48	32	22	12	30	101	26	320	3710
1997	35	11	53	7	9	3	31	63	66	278	4150
1998	14	27	43	15	3	16	74	91	53	336	3900
1999	56	64	19	20	73	21	75	49	87	464	3560
2000	18	77	75	6	19	45	78	50	13	381	3840
2001	4	22	63	35	16	68	51	8	129	396	4730
2002	5	34	7	10	33	31	25	60	46	251	3680
2003	35	29	26	32	31	6	21	56	35	271	3050
2004	90	22	21	40	35	59	41	23	76	407	5500
2005	36	70	26	11	31	15	86	61	83	419	4490
2006	12	16	63	25	55	52	160	76	75	534	3860
2007	26	17	4	21	31	27	59	56	60	301	3386
2008	37	40	38	25	8	41	60	61	83	393	5167
2009	25	23	60	26	32	38	5	16	103	328	3930
	Mean air temperature (°C)									X	kg ha^{-1}
1990	10.6	3.6	0.5	–0.8	4.2	8.5	10.7	16.1	18.2	8.0	5480
1991	10.2	5.6	0.4	–0.5	–2.6	7.2	9.5	12.7	19.1	6.8	5770
1992	9.5	5.7	–4.2	–2.2	0.7	5.3	11.5	15.9	20.0	6.9	4130
1993	9.8	5.4	–1.1	–1.6	–3.3	3.0	10.1	18.8	19.3	6.7	3120
1994	11.9	1.9	2.7	2.4	1.6	7.3	11.5	15.7	19.2	8.2	5300
1995	9.3	4.3	0.4	–1.8	4.6	5.5	10.0	15.3	18.8	7.4	4180
1996	11.8	2.2	–0.2	–2.5	–3.7	0.8	11.8	17.8	20.1	6.5	3710
1997	10.8	7.3	–1.7	–2.6	0.5	4.2	7.1	16.5	19.4	6.8	4150
1998	8.9	6.7	2.3	2.6	3.9	3.9	12.8	15.8	19.9	8.5	3900
1999	10.8	2.5	–5.5	–1.2	–1.5	6.4	11.9	15.4	20.5	6.6	3560
2000	10.5	3.5	0.3	–3.5	2.2	4.9	14.0	17.9	20.6	7.8	3840
2001	13.1	8.5	2.2	0.6	2.3	7.6	10.8	17.7	17.6	8.9	4730
2002	12.7	2.0	–5.3	–1.3	4.2	7.2	11.3	18.9	20.4	7.8	3680
2003	9.9	6.6	–1.8	–3.3	–5.8	3.6	10.0	19.7	21.7	6.7	3050
2004	8.2	6.8	–0.1	–3.3	–0.7	4.8	11.4	14.8	19.3	6.8	5500
2005	11.1	4.9	0.9	–0.9	–3.7	2.2	10.8	16.2	18.4	6.7	4490
2006	10.8	3.5	0.2	–3.4	–1.4	3.2	12.1	15.4	18.6	6.6	3860
2007	11.3	6.2	2.2	3.7	4.1	9.1	12.6	18.4	22.2	10.0	3386
2008	9.7	3.5	–0.6	1.0	3.0	6.2	11.4	16.8	20.6	8.0	5167
2009	10.8	6.5	3.1	–1.0	1.3	5.4	14.9	17.4	19.8	8.7	3930
	Long-term mean (Debrecen, 1961–1990)										
mm	32	47	46	39	32	36	45	61	83	420	
°C	10.3	4.5	–0.2	–2.6	0.2	5.0	10.7	15.8	18.7	6.9	

Year = year of harvest; Oct.–Dec. previous year

Table 5
Precipitation and mean air temperatures (data from the Osijek meteorological station) and wheat grain yields in the eastern Croatia region

Year	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Σ	Yield kg ha^{-1}
	Precipitation (mm)										
1990	49	42	18	12	39	26	38	26	101	351	6500
1991	34	53	59	29	30	37	79	102	26	449	5680
1992	–	–	–	–	–	13	59	40	112	–	4740
1993	155	105	50	23	9	62	43	48	69	564	4840
1994	43	96	93	45	31	34	52	35	88	517	4520
1995	58	16	45	71	53	44	52	96	106	541	4390
1996	6	54	104	31	51	42	82	78	30	478	4210
1997	61	99	67	44	43	23	59	38	86	520	4570
1998	100	42	92	91	1	21	54	49	26	476	4800
1999	97	69	30	36	61	29	45	89	150	606	3620
2000	22	124	98	18	15	41	28	26	10	382	5000
2001	10	42	37	75	23	83	72	60	240	642	4700
2002	5	74	34	11	48	10	64	135	37	418	4730
2003	59	40	24	66	16	5	12	18	44	284	3300
2004	132	45	27	50	50	41	137	7	77	566	–
2005	94	115	42	36	66	34	55	46	112	600	4310
2006	4	16	111	33	50	53	87	79	91	524	4760
2007	31	33	6	25	47	76	3	56	33	310	4920
2008	92	103	48	33	5	85	50	67	76		
2009	30	48		60	29	26	19	39	63		
	Mean air temperature (°C)									X	kg ha^{-1}
1990	11.4	5.0	2.3	0.5	6.1	9.5	11.2	17.6	19.5	9.2	6500
1991	12.2	6.4	1.0	1.1	–2.6	8.7	9.7	12.9	20.0	7.7	5680
1992	–	–	–	–	–	6.5	12.1	17.1	19.9	–	4740
1993	11.0	7.1	0.6	0.6	–1.1	4.8	11.9	19.0	20.0	8.6	4840
1994	12.9	1.9	2.3	2.3	2.0	1.5	9.2	11.8	17.0	8.8	4520
1995	9.9	7.1	1.8	1.8	6.1	5.4	11.7	1,5.5	18.2	8.5	4390
1996	12.1	3.6	1.4	–1.2	–2.4	2.9	11.5	18.0	21.1	7.4	4210
1997	11.5	8.2	–0.3	–1.3	3.5	6.0	7.9	17.8	20.8	8.2	4570
1998	8.9	6.2	2.8	2.8	5.0	4.8	12.6	16.2	21.4	9.0	4800
1999	12.3	4.0	–3.3	0.4	1.1	8.2	12.6	17.3	20.3	8.1	3620
2000	11.7	4.0	0.7	–1.6	4.2	7.0	14.9	18.4	22.5	9.1	5000
2001	14.1	10.0	3.0	2.7	4.4	9.9	10.9	18.4	18.1	10.1	4700
2002	13.9	3.5	–3.8	–0.2	6.0	8.4	11.2	18.6	21.1	8.7	4730
2003	11.3	8.8	0.9	–2.4	–3.1	5.9	11.3	20.1	24.3	8.6	3300
2004	9.4	7.5	1.4	–1.4	2.3	5.8	11.7	14.6	19.2	7.8	–
2005	13.2	6.2	1.9	0.0	–3.2	4.1	11.5	17.0	19.5	7.8	4310
2006	11.7	5.0	1.7	–1.6	1.1	5.3	12.7	16.2	20.1	8.0	4760
2007	13.0	7.8	3.0	5.8	6.1	8.5	13.3	18.2	22.3	10.9	4920
2008	10.3	4.0	0.1	1.5	4.9	7.5	12.5	18.1	21.5		
2009	13.0	7.5		–1.1	2.3	6.8	14.6	18.3	19.2		
	Long-term mean (Osijek, 1961–1990)										
mm	41	57	52	47	40	45	54	58	88	482	
°C	11.2	5.4	0.9	–1.2	1.6	6.1	11.3	16.5	19.5	7.9	

Year = year of harvest; Oct.–Dec. previous year

Table 6
Pearson correlation analysis on winter wheat yields and meteorological parameters in four counties of Eastern Hungary (1990–2009)

Counties	Rainfall			Average temperature	
	Winter half-year	Spring period	Vegetation period	Spring period	Vegetation period
Hajdú-Bihar (HB)	0.111	0.054	0.118	–0.321	0.048
Jász-Nagykun-Szolnok (JNS)	–0.171	0.451*	0.158	–0.424	0.024
Szabolcs-Szatmár-Bereg (SSB)	0.034	0.171	0.130	–0.322	0.002
Békés (B)	–0.238	0.429	0.209	–0.245	0.148

*Correlation significant at the 0.05 level

References

Baginskas, B. P., Zhyamaitis, A. B., Kuchinskas, I. M. (1985): Effect of fertilizers on yield of winter wheat under different meteorological conditions. *Byulleten Vsesoyuznogo Nauchno issledovatelskogo Instituta Udobrenii i Agropochvovedeniya*, **72**, 29–32.

Balogh, Á., Pepó, P. (2008): Cropyear effects on the fertilizer responses of winter wheat (*Triticum aestivum* L.) genotypes. *Cereal Res. Commun.*, **36**, 732–734.

Berzsenyi, Z. (1993): *Növényanalízis a kukoricatermesztési kutatásokban.* (Plant analysis in maize production research.) DSc Thesis, Martonvásár.

Birkás, M., Dexter, A. R., Kalmár, T., Bottlik, L. (2006): Soil quality – soil condition – production stability. *Cereal Res. Commun.*, **34**, 135–138.

Domitruk, D. R., Duggan, B. L., Fowler, D. B. (2000): Soil water use, biomass accumulation and grain yield of no-till winter wheat on the Canadian prairies. *Can. J. Plant Sci.*, **80**, 729–738.

Janekovic, G. (1971): *Zbornik radova Prvog znanstvenog sabora Slavonije i Baranje* (Pedologic characteristics of Slavonia and Baranya). Osijek, Yugoslavia. pp. 115–176.

Jolánkai, M. (1982): *Őszi búzafajták tápanyag- és vízhasznosítása.* (Nutrient and water utilisation of winter wheat varieties.) PhD thesis, Martonvásár.

Josipovic, M., Kovacevic, V., Petosic, D., Sostaric, J. (2005): Wheat and maize yield variations in the Brod-Posavina area. *Cereal Res. Commun.*, **33**, 229–233.

Kosminski, C., Borin, M., Attin, M. (1994): Climatic risk to crops in Poland. In: *Proceedings of the Third Congress of the European Society for Agronomy*, Padova University, Abano-Padova, Italy, 18–22 September 1994. pp. 818–819.

Kovacevic, V. (2005): Wheat yield variations among the years in the Eastern Croatia. In: *Proceedings of the XL Croatian Symposium on Agriculture with International Participation*, 15–18 February 2005, Opatija, Croatia, pp. 453–454.

Kovacevic, V., Josipovic, M. (1995): Winter wheat (*Triticum aestivum* L.) yield variations in Croatia from 1960 to 1994. *Fragmenta Agronomica*, **46**, 28–29.

Kovacevic, V., Josipovic, M., Sostaric, J., Iljkic, D., Markovic, M. (2009): Wheat yield variations among years in eastern Croatia with emphasis on Vukovar-Syrmium area. In: *Proceedings of 2nd International Scientific/Professional Conference "Agriculture in Nature and Environment Protection"*, Vukovar 4th–6th June 2009, pp. 115–121.

Marijanovic, M., Markulj, A., Tkalec, M., Jozic, A., Kovacevic, V. (2010): Impact of precipitation and temperature on wheat (*Triticum aestivum* L.) yields in Eastern Croatia. *Acta Agriculturae Serbica*, **XV** (**30**), 117–123.

Olsen, J. E., Bindi, M. (2002): Consequences of climate change for European agricultural productivity, land use and policy. *Eur. J. Agron.*, **16**, 239–262.

Pepó, P. (2004a): *Őszi búza tápanyagellátása a Hajdúságban.* (Nutrient supplies to winter wheat in the Hajdúság region.) DSc thesis, Debrecen.

Pepó, P. (2004b): Az évjárat hatása az őszi búza termésmennyiségére tartamkísérletben. (Effect of year on the yield of winter wheat in long-term experiments.) *Növénytermelés*, **53**, 339–350.

Pepó, P. (2009a): A növénytermesztés aktuális kérdései. (Current questions in crop production.) pp. 17–20. In: Harcsa M. (ed.), *V. Növénytermesztési Tudományos Nap.* Növénytermesztés: Gazdálkodás-Klímaváltozás-Társadalom, Keszthely, 2009. november 19. Akadémiai Kiadó, Budapest, ISBN: 978 963 05 8804 1

Pepó, P. (2009b): Effects of water supply as an abiotic stress on the yields and agronomic traits of winter wheat (*Triticum aestivum* L.) on chernozem soil. *Cereal Res. Commun.*, **37**, 29–32.

Pepó, P. (2009c): Hatékony búzatermesztés. (Efficient wheat production.) *Magyar Mezőgazdaság*, **64**, 14–16.

Ruzsányi, L. (1991): A növények elővetemény-hatásának értékelése vízháztartási szempontból. (Evaluating the forecrop-effect of different plants from the point of view of water-budget.) *Növénytermelés*, **40**, 71-77.

Shen, S. H., Gao, W. Y., Li, B. B. (1999): Water consumption and its impact on yield of winter wheat in Xifeng. *J. Nanjing Inst. Meteorology*. **22**, 88–94.

Škorić, A. (1977): Tla Slavonije i Baranje. pp. 7–58. In: Škorić, A. (ed.), *Tla Slavonije i Baranje.* Izdavački zavod Jugoslavenske Akademije, Zagreb, Yugoslavia.

Vad, A., Dóka, L. (2009): Cropyear as abiotic stress effect on the yields of maize (*Zea mays* L.) in different crop rotation. *Cereal Res. Commun.*, **37**, 253–256.

Várallyay, G. (2007): A globális klímaváltozás. A VAHAVA Jelentés. (Global climate change. The VAHAVA report.) *Agrokémia és Talajtan*, **56**, 199–202.

Zhao, J. B. (1987): Evaluation on the water condition of winter wheat and reasonable use of water resources in Cangzhou region, Hebei Province. *Meteorological Monthly*, **13**, 23–27.

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ANALYSIS OF THE EFFECT OF N FERTILISATION ON THE GROWTH DYNAMICS OF WINTER WHEAT VARIETIES USING THE HUNT–PARSONS MODEL

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The effect of nitrogen (N) fertilisation on the growth of winter wheat varieties was examined in three diverse years using the functional method of growth analysis. The main plot in the two-factorial, split-plot experiment was the N treatment and the subplot the variety. The wheat varieties Mv Toborzó (extra-early), Mv Palotás (early) and Mv Verbunkos (mid-early) were treated with N rates of 0, 80, 160 and 240 kg N ha^{-1} (N_0, N_{80}, N_{160}, N_{240}). The Hunt–Parsons (HP) program fitted a third-degree exponential function to the dry matter and leaf area data. In 2007 and 2008 dry matter accumulation continued up to the N_{240} rate and in 2009 to the N_{160} rate. In all three years the highest value was recorded for Mv Verbunkos (4.62 g $plant^{-1}$ in 2007, 4.63 g in 2008 and 4.51 g in 2009). The highest value of maximum leaf area (237.5 cm^2) was found for Mv Verbunkos in the N_{240} treatment. The maximum values of leaf area in each N treatment, averaged over years and varieties (cm^2 $plant^{-1}$), were as follows: N_0: 86.2; N_{80}: 141.0; N_{160}: 164.0; N_{240}: 173.1. The parameter AGR_{mean} exhibited the highest value (8.04 g day^{-1} 10^{-2}) in the N_{160} treatment, while among the varieties Mv Verbunkos had the highest mean value (7.18 g day^{-1} 10^{-2}). The highest value of RGR_{mean} was achieved by Mv Toborzó in the N_{160} treatment in 2009 (3.94 g g^{-1} day^{-1} 10^{-2}). The value of NAR_{mean} increased up to fertiliser rates of N_{160} and N_{240}, with mean values (g m^{-2} day^{-1}) of N_0: 2.35, N_{80}: 2.44, N_{160}: 2.53 and N_{240}: 2.47. The highest value of NAR (3.29 g m^{-2} day^{-1}) was obtained for Mv Palotás in the N_{160} treatment in 2008. On average the greatest value of LAR_{max} was recorded in the N_{160} treatment (172.8 cm^2 g^{-1}), while the highest absolute value (213.6 cm^2 g^{-1}) was achieved by Mv Toborzó in 2008. The unfavourable effect of the drought in 2007 was clearly reflected in the values of the growth parameters.

Key words: winter wheat, N fertilisation, growth analysis, Hunt–Parsons model, growth parameters

Introduction

The basic principles and classical method of growth analysis were detailed by Evans (1972), while the functional method of growth analysis was elaborated by Causton and Venus (1981) and Hunt (1982). The latter method involves the fitting of mathematical functions to the measurement data, which are then used to calculate the instantaneous values of various parameters. The primary

variables used in growth analysis are dry mass and leaf area data recorded at various intervals. The functional method has numerous advantages compared with classical growth analysis, as summarised by Hunt (1982). One advantage is that the functional method considers the data from all the sampling dates simultaneously, while the classical method uses various formulae to calculate the parameters from the data of only two sampling dates. A further advantage of the functional method is that it balances out small random fluctuations, making the complex process of growth easier to interpret. Hunt and Parsons (1974) developed a growth analysis program which uses the stepwise method to choose the type of function that best fits the data. This growth analysis program is widely used, both in Hungary and throughout the world. Reviews of the functional method of growth analysis and of its application in Hungary were published by Berzsenyi (2000; 2002). Sugár and Berzsenyi (2009) used the classical method of growth analysis to investigate the effect of N fertilisation on the growth dynamics and yield of various wheat varieties. Micskei et al. (2010) analysed the effect of farmyard manure and mineral fertiliser on the growth of maize in a long-term experiment, using the Hunt–Parsons program for plant growth analysis. Berzsenyi (1996) studied the N fertiliser responses of maize hybrids with the help of growth analysis, while Árendás et al. (2010) demonstrated the fertiliser responses of maize and winter wheat as a function of year and forecrop in a long-term experiment.

The aim of the present work was to investigate the effect of nitrogen fertilisation on the growth of different winter wheat varieties in several years using the Hunt–Parsons model. The first results of this work were published by Sugár and Berzsenyi (2010).

Materials and methods

Growth analysis was carried out in the 2006/2007, 2007/2008 and 2008/2009 seasons in a long-term crop rotation experiment involving winter wheat. The experiment was set up in 1980 in a two-factorial, split-plot design with four replications. The main plots included eight N treatments (in 40 kg ha^{-1} steps from 0–280 kg ha^{-1}), and the subplots 12 wheat varieties. The subplots measured 13.5 m^2. The growth analysis was performed on four N treatments (N_0, N_{80}, N_{160}, N_{240}) and three wheat varieties with different maturity periods: Mv Toborzó (extra-early), Mv Palotás (early) and Mv Verbunkos (mid-early). When sown in early to mid-October, the mean heading dates are May 13th for Mv Toborzó, May 20th for Mv Palotás and May 22nd for Mv Verbunkos.

Of the years included in the study, 2006/2007 was extremely dry. The rainfall sum (200 mm) was around a third of that recorded in the other two years (638 mm in 2008 and 617 mm in 2009) and was well below the 30-year mean (513 mm). There was no significant difference between the years in terms of daily mean temperature (2007: 12°C; 2008 and 2009: 10°C).

For the purpose of growth analysis, destructive samples consisting of 5 plants per plot were taken once a week on a total of 25 occasions in 2007, 21 in 2008 and 17 in 2009, covering the whole growing season. Sampling was begun when the wheat reached the two-leaf stage. The dry mass of the samples was determined after drying for 48 h in a drying cabinet at 60°C and the fresh leaf area was recorded using an AM300 leaf area meter. The method elaborated by Hunt and Parsons (1974) was used to evaluate the data. The Hunt–Parsons program fits first-, second- or third-degree exponential functions to the basic dry mass and leaf area data in a stepwise manner. The program also calculates the standard error and the values of the 95% confidence interval for the whole of the sampling period. The absolute growth rates (AGR, ALGR), relative growth rates (RGR, RLGR), net assimilation rate (NAR) and leaf area ratio (LAR) calculated by the program were characterised in terms of dynamics over time and mean and maximum values.

Results and discussion

Effect of N fertilisation on the dynamics of dry matter production and leaf area of winter wheat varieties

The Hunt–Parsons program fitted third-degree exponential functions to the dry matter accumulation data (Figs. 1–3), with the exception of the dry matter accumulation of Mv Toborzó and Mv Verbunkos in the N_0 treatment in 2007, for which second-degree functions were fitted. These second-degree exponential functions were only plotted up to the maximum values of the data. In all cases the functions gave a good fit to the measurement data (R^2 = 94.7–99.3%). The dynamics of dry matter accumulation was of the sigmoid type up to the maximum value and gave a good expression of the effect of the nitrogen treatments.

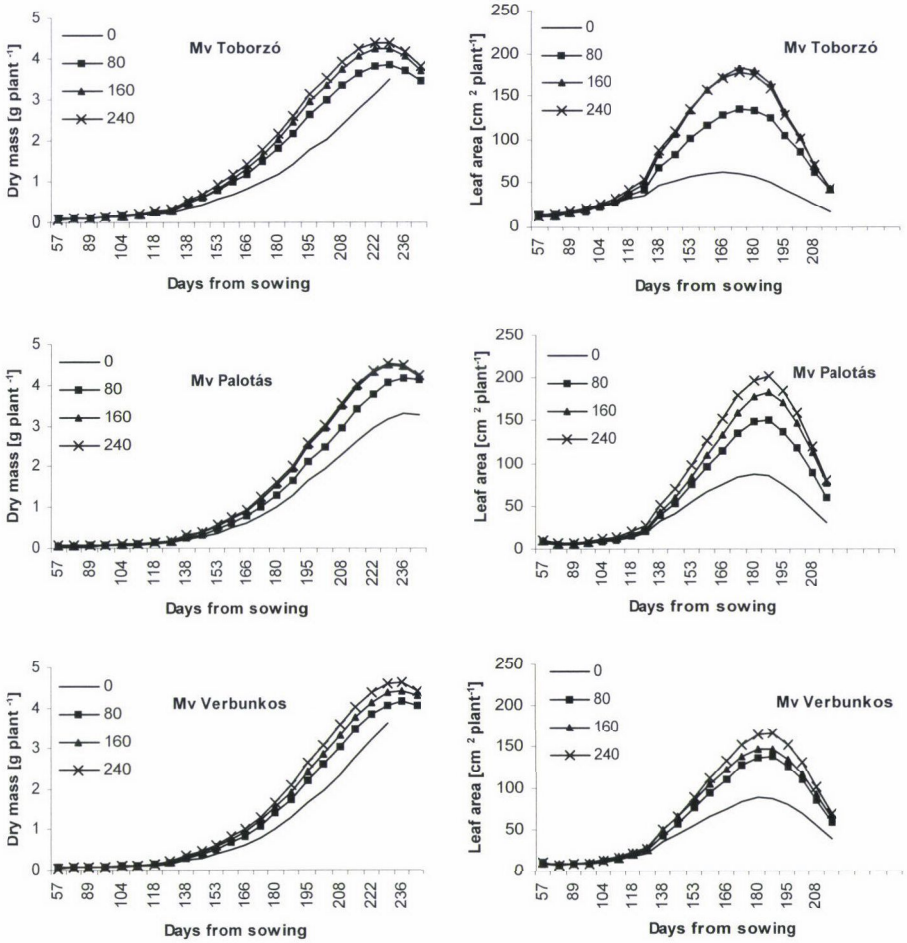

Fig. 1. Effect of N fertilisation and genotype on the dynamics of dry matter accumulation and leaf area in 2007

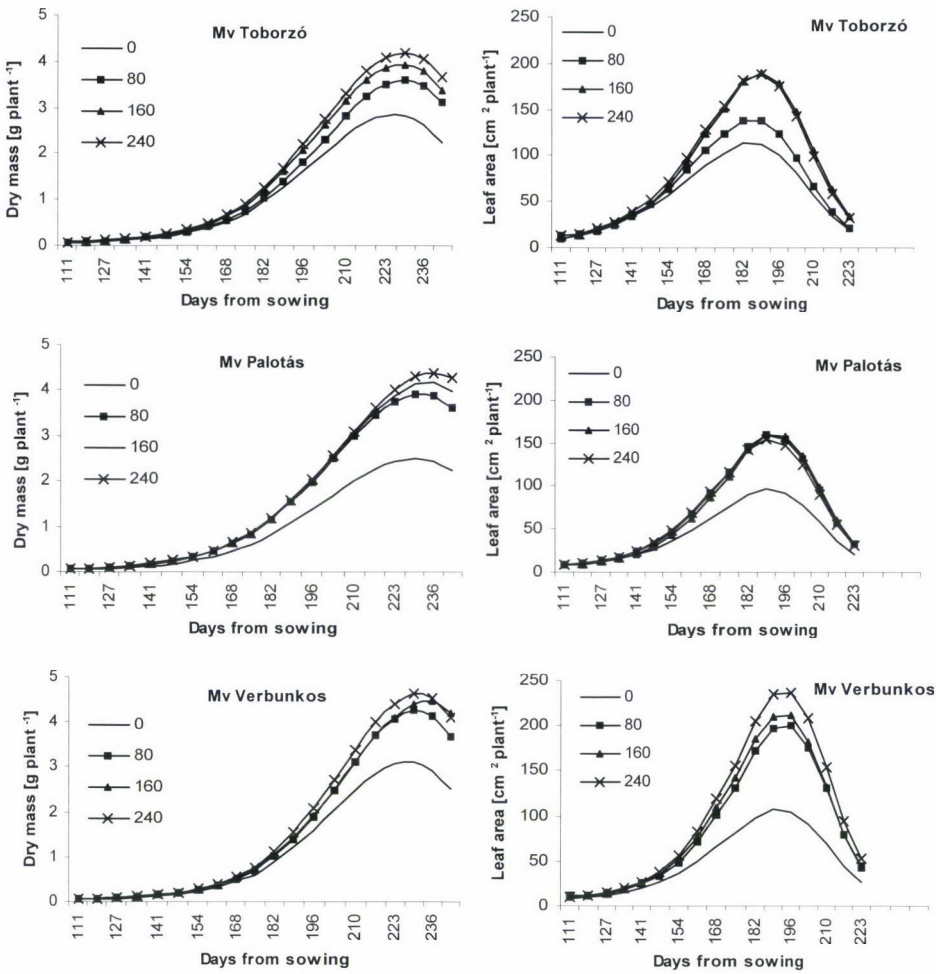

Fig. 2. Effect of N fertilisation and genotype on the dynamics of dry matter accumulation and leaf area in 2008

In response to N fertilisation, the dry matter production increased up to the N_{240} rate in 2007 and 2008 and up to the N_{160} rate in 2009. Averaged over the varieties and years the following values were obtained for the individual N treatments: N_0: 2.88, N_{80}: 3.96, N_{160}: 4.33 and N_{240}: 4.33 g plant^{-1}. In all three years the maximum values were recorded for Mv Verbunkos (2007: 4.62 g, 2008: 4.63 g, 2009: 4.51 g plant^{-1}). The dynamics of dry matter production over time reflected the diverse maturity dates of the wheat varieties. Depending on the treatment and year, the number of days after sowing when the maximum values were recorded was 228–231 for Mv Toborzó, 233–239 for Mv Palotás and 232–241 for Mv Verbunkos.

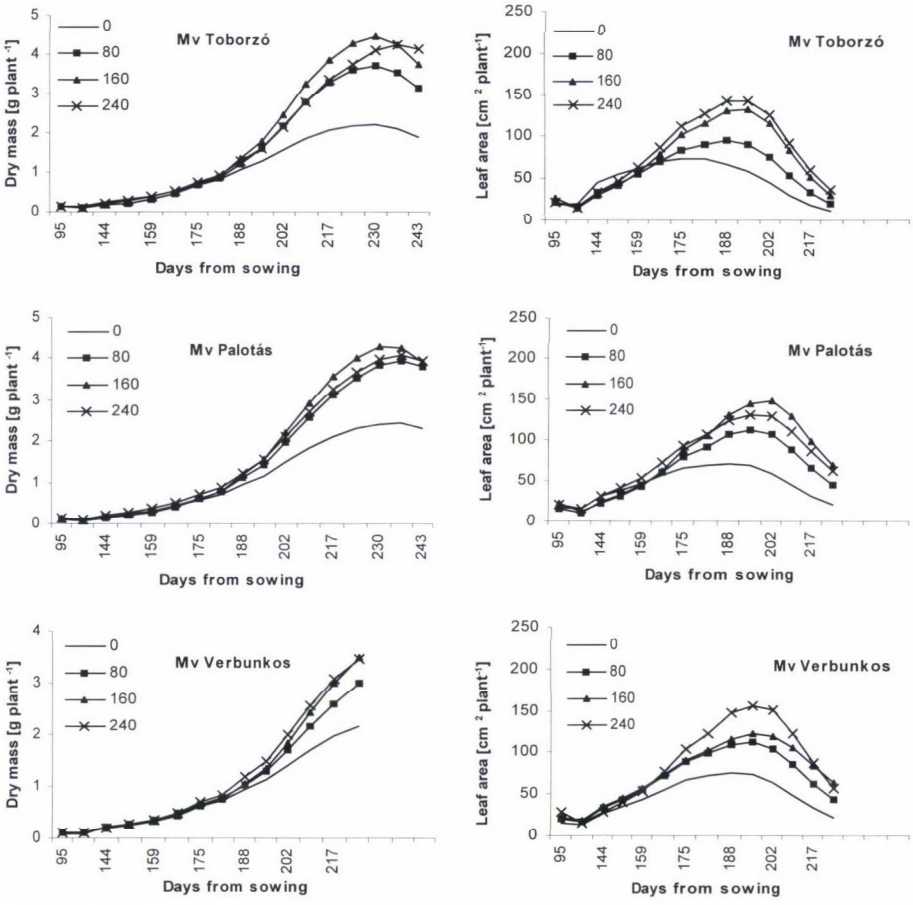

Fig. 3. Effect of N fertilisation and genotype on the dynamics of dry matter accumulation and leaf area in 2009

The HP program described the leaf area dynamics with a third-degree curve (R^2 = 85.9–96.4%) in all cases (Figs. 1–3). Depending on the variety and year, the leaf area values rose up to the N_{160} or N_{240} level. The maximum values of leaf area for the individual N fertiliser rates, averaged over year and variety, were as follows (cm^2 $plant^{-1}$): N_0: 86.2, N_{80}: 141.0, N_{160}: 164.0, N_{240}: 173.1. The highest value of maximum leaf area (237.5 cm^2) was recorded for Mv Verbunkos in the N_{240} treatment in 2008. This variety also exhibited the highest value averaged over years and N treatments (146.9 cm^2). Averaged over varieties and N treatments, the maximum leaf area per plant in 2009 (114.5 cm^2) was considerably lower than in the other two years (2007: 143.4 cm^2, 2008: 167.3 cm^2). The dynamics of leaf area followed the maturity time of the varieties. Depending on the year, the maximum was achieved on the 171–187th day by Mv Toborzó, on the 181–195th day by Mv Palotás and on the 180–194th day by Mv Verbunkos.

Effect of N fertilisation on the growth rates of total dry matter (AGR, RGR) and leaf area (ALGR, RLGR) of the wheat varieties

The dynamics of the absolute growth rate (AGR) was typically described by a Gauss curve. The AGR dynamics of the N_0 treatment differed greatly from that of the fertilised treatments. In all three years the AGR dynamics was similar, with differences mainly in the maximum values. The AGR dynamics in 2008 is illustrated in Figure 4, and the maximum AGR values (AGR_{max}) in Table 1. The differences in AGR between N_0 and the other N treatments were particularly evident in 2008 and 2009. Averaged over varieties and years, the AGR_{max} values were lowest (5.01 g day^{-1} 10^2) in the N_0 treatment, increasing in the N_{80} treatment (7.11 g day^{-1} 10^2) and reaching a maximum in the N_{160} treatment (8.04 g day^{-1} 10^2), with a reduction in the N_{240} treatment (7.75 g day^{-1} 10^2). Averaged over N treatments and years, Mv Verbunkos exhibited the highest absolute growth rate (7.18 g day^{-1} 10^2). The most favourable year for AGR was 2008, with a maximum value of 7.44 g day^{-1} 10^2, averaged over N treatments and varieties, while the lowest AGR_{max} value was obtained in 2007 (6.48 g day^{-1} 10^2). The highest values of AGR_{max} were recorded for Mv Palotás in 2007 (7.56 g day^{-1} 10^2), for Mv Verbunkos in 2008 (9.42 g day^{-1} 10^2) and for Mv Toborzó in 2009 (9.65 g day^{-1} 10^2). The AGR_{max} values for Mv Toborzó and Mv Palotás were highest in 2009 (9.65 and 9.41 g day^{-1} 10^2, respectively) and for Mv Verbunkos in 2008 (9.42 g day^{-1} 10^2).

Table 1

Effect of N fertiliser on the maximum values of absolute growth rate (AGR) and the mean values of relative growth rate (RGR) of the wheat varieties

Wheat variety	Year	N fertiliser treatment (kg ha^{-1})			
		0	80	160	240
		AGR_{max} (g day^{-1} 10^2)			
Toborzó	2007	5.37	5.70	6.49	6.60
	2008	5.59	7.08	7.65	8.11
	2009	3.77	7.68	9.65	7.98
Palotás	2007	5.13	6.61	7.56	7.52
	2008	4.56	7.32	7.58	7.75
	2009	4.26	7.86	9.41	7.80
Verbunkos	2007	6.17	6.44	6.91	7.28
	2008	6.48	8.89	8.82	9.42
	2009	3.76	6.49	8.26	7.29
		RGR_{mean} (g g^{-1} day^{-1} 10^2)			
Toborzó	2007	2.34	2.45	2.59	2.54
	2008	2.94	3.20	3.44	3.39
	2009	2.68	3.69	3.94	3.47
Palotás	2007	2.73	2.82	2.82	2.73
	2008	3.15	3.36	3.53	3.53
	2009	2.87	3.54	3.79	3.33
Verbunkos	2007	2.72	2.72	2.75	2.84
	2008	3.28	3.37	3.62	3.52
	2009	2.71	3.12	3.48	3.31

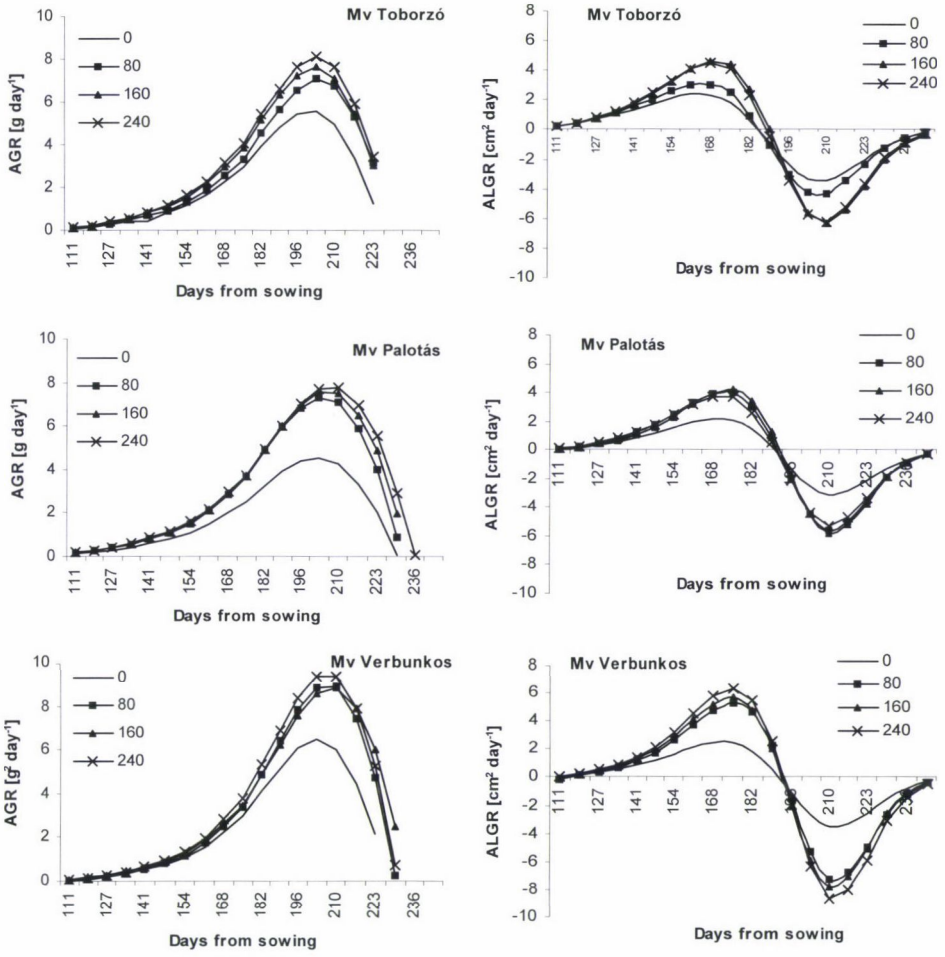

Fig. 4. Effect of N fertilisation and genotype on the dynamics of absolute growth rate of total dry matter (AGR) and the absolute growth rate of leaf area (ALGR) in 2008

Table 1 shows the mean relative growth rate (RGR) of the total dry matter production per plant up to the end of dry matter accumulation, which occurred at around the end of May in all three years. The RGR values were the smallest in the N_0 treatment, rising with the N rate up to N_{160} and declining again at N_{240}. The only exception was Mv Verbunkos in 2007, when the RGR values did not differ significantly in the different N treatments. The mean RGR values were lower in 2007 than in the other two years. The highest values were obtained in the N_{160} treatment in 2009 for Mv Toborzó and Mv Palotás (3.94 and 3.79 g g^{-1} day^{-1} 10^2). In the case of Mv Verbunkos, very similar maximum values were recorded in 2008 and 2009, again in the N_{160} treatment (3.62 and 3.48 g g^{-1} day^{-1} 10^2). Overall, the highest RGR_{mean} value was observed for Mv Toborzó in the N_{160} treatment in 2009 (3.94 g g^{-1} day^{-1} 10^2).

The dynamics of the absolute growth rate of the leaf area (ALGR) was characterised by an increase up to a maximum value, followed by a gradual decline until the end of the growth period. The rapid withering of the foliage resulted in a negative growth rate, increasing up to a negative maximum, after which the withering rate declined. The dynamics of ALGR was similar for all the experimental factors. The ALGR dynamics for 2008 is illustrated in Figure 4, and the maximum values, which exhibited substantial differences, in Table 2. Averaged over years and varieties, the maximum values of ALGR per plant in the individual N treatments were as follows: N_0: 1.66, N_{80}: 3.00, N_{160}: 3.69 and N_{240}: 3.89 cm^2 day^{-1}. For all three varieties the highest ALGR values were obtained in 2008 (Mv Toborzó: 4.6, Mv Palotás: 4.18, Mv Verbunkos: 6.36 cm^2 day^{-1}). The highest value recorded in the experiment was thus found for Mv Verbunkos in the N_{240} treatment in 2008 (6.36 cm^2 day^{-1}).

As can be seen in Table 2, the mean value of the relative leaf area growth rate per plant (RLGR) until the maximum leaf area was achieved increased with the N rate up to N_{160}. The only exception was Mv Verbunkos, where the mean RLGR continued to rise in all three years up to N_{240}. In all four treatments Mv Toborzó had lower RLGR values in 2007 than in 2008 or 2009, while the mean relative growth rate of Mv Verbunkos was higher in all the treatments in 2008 than in the other two years. The RLGR values were the lowest in 2007, except for Mv Verbunkos in the N_{0-160} treatments. The highest mean values for all the varieties were recorded in 2007 and 2009 by Mv Palotás in the N_{160} treatment (3.17 and 4.22 cm^2 cm^{-2} day^{-1}) and in 2008 by Mv Verbunkos in the N_{240} treatment (4.09 cm^2 cm^{-2} day^{-1}).

Table 2

Effect of N fertiliser and the wheat variety on the maximum value of absolute growth rate (ALGR) and the mean value of relative leaf area growth rate (ALGR)

Wheat variety	Year	N fertiliser treatment (kg ha^{-1})			
		0	80	160	240
		$ALGR_{max}$ (cm^2 day^{-1})			
Toborzó	2007	0.81	2.31	3.45	3.23
	2008	2.38	3.07	4.60	4.51
	2009	1.19	1.91	3.00	3.16
Palotás	2007	1.69	3.19	3.92	4.26
	2008	2.18	3.98	3.93	3.73
	2009	1.21	2.44	3.55	2.70
Verbunkos	2007	1.54	2.72	2.82	3.38
	2008	2.46	5.28	5.67	6.36
	2009	1.45	2.14	2.28	3.64
		$RLGR_{mean}$(cm^2 cm^{-2} day^{-1})			
Toborzó	2007	1.57	2.48	2.92	2.80
	2008	2.90	3.22	3.81	3.55
	2009	1.54	2.96	3.87	3.80
Palotás	2007	2.58	3.10	3.17	3.13
	2008	2.87	3.43	3.53	3.48
	2009	2.13	3.58	4.22	3.31
Verbunkos	2007	2.38	2.86	2.82	3.05
	2008	3.26	3.73	3.91	4.09
	2009	2.20	2.59	2.65	3.59

Effect of N fertilisation on the net assimilation rate (NAR) and leaf area ratio (LAR) of wheat varieties

The dynamics of the net assimilation rate (NAR) was characterised by a rapid growth stage up to a relatively constant value, followed by a further rapid increase as the foliage withered. The NAR dynamics recorded in 2008 is illustrated in Figure 5. The value of NAR was constant for a relatively long period in 2007 and 2008, from approximately the 110th to the 180th day after sowing, i.e. until first node appearance. The mean NAR values during this period are summarised in Table 3.

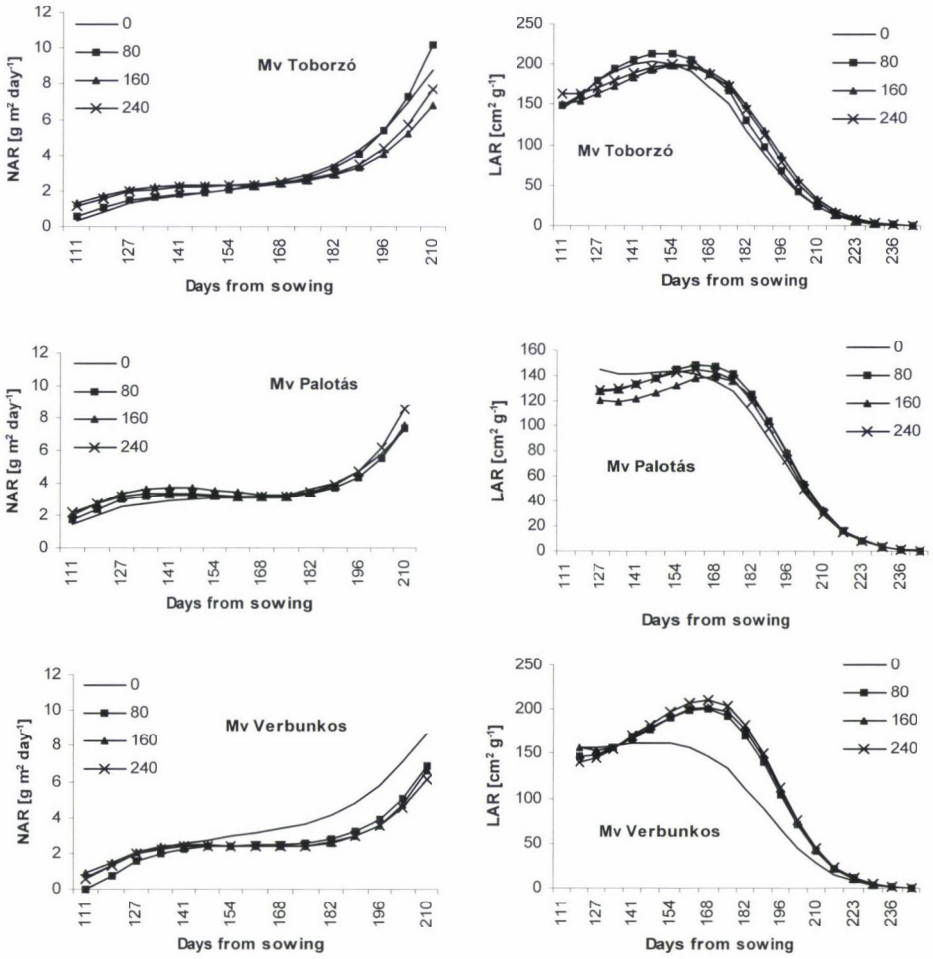

Fig. 5. Effect of N fertilisation and genotype on the net assimilation rate (NAR) and leaf area ratio (LAR) in 2008

Table 3
Effect of N fertilisation on the mean value of net assimilation rate (NAR) and the maximum value of leaf area ratio (LAR)

Wheat variety	Year	N fertiliser treatment (kg ha^{-1})			
		0	80	160	240
		NAR_{mean} (g m^{-2} day^{-1})			
	2007	1.76	2.07	1.98	2.04
Toborzó	2008	1.70	1.80	2.18	2.09
	2009	2.53	2.99	2.91	2.55
	2007	2.22	2.44	2.49	2.22
Palotás	2008	2.73	2.94	3.29	3.10
	2009	2.82	3.11	3.19	2.90
	2007	1.97	2.32	2.21	2.58
Verbunkos	2008	2.48	1.89	2.16	2.06
	2009	2.91	2.43	2.40	2.68
		LAR_{max} (cm^2 g^{-1})			
	2007	148.1	146.8	172.2	163.0
Toborzó	2008	204.2	213.6	198.9	200.4
	2009	188.5	191.6	183.4	164.6
	2007	148.7	159.8	158.7	172.8
Palotás	2008	143.1	148.3	139.0	144.9
	2009	165.5	150.2	157.5	152.3
	2007	147.8	147.7	154.0	144.7
Verbunkos	2008	163.0	200.0	202.8	210.4
	2009	128.1	155.4	188.6	157.2

As this parameter expresses the dry matter gain per unit leaf area, the value of NAR exhibits an increase as the leaf area declines. Mean NAR values rose with increasing N rates up to N_{160} or N_{240}. Averaged over varieties and years the following values were observed for the individual N treatments (g m^{-2} day^{-1}): N_0: 2.35, N_{80}: 2.44, N_{160}: 2.53 and N_{240}: 2.47. In the case of Mv Verbunkos the mean NAR was highest in the N_0 treatment in 2008 and 2009. This could have been due to the fact that the stand was much thinner on plots without N fertiliser, resulting in greater assimilation due to the better light conditions. The highest value of NAR (3.29 g m^{-2} day^{-1}) was obtained for Mv Palotás in the N_{160} treatment in 2008. Among the years, the lowest NAR values were obtained in 2007 and the highest in 2008 and 2009 (3.29 g m^{-2} day^{-1} and 3.19 g m^{-2} day^{-1} for Mv Palotás). The dynamics of leaf area ratio (LAR) over time was used to characterise the leaf area per plant dry mass. The dynamics recorded in 2008 is presented in Figure 5. After a short initial period of growth, LAR reached its maximum value, after which it tended to decline until the end of the vegetation period. The maximum values (Table 3) were obtained earlier in 2007 (between days 138 and 153) than in the other two years (days 154–175). There were also differences in dynamics between the varieties, with Mv Toborzó exhibiting maximum LAR approximately a week earlier than the other varieties. The maximum values differed with the N rate, being lower in all cases at the N_0 level. Averaged over varieties and years the LAR_{max} values in the individual N treatments were

($cm^2\ g^{-1}$): N_0: 159.7, N_{80}: 168.2, N_{160}: 172.8 and N_{240}: 167.8. The highest LAR_{max} value (213.6 $cm^2\ g^{-1}$) was obtained for Mv Toborzó in the N_{80} treatment in 2008, with an only slightly lower value (210.4 $cm^2\ g^{-1}$) for Mv Verbunkos in the N_{240} treatment, again in 2008.

In conclusion it can be stated that the values of dry matter production, leaf area and growth parameters rose in most cases up to N_{160} or N_{240}. Among the varieties, Mv Verbunkos had the greatest dry matter production in all three years, and also exhibited the highest values of leaf area, AGR_{max} and $ALGR_{max}$. The highest LAR was achieved by Mv Toborzó and the highest RLGR and NAR by Mv Palotás. Of the years, 2007 was extremely dry, as reflected in the growth parameters and yield. The highest values of leaf area, AGR, ALGR, RLGR, NAR and LAR were obtained in 2008.

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References

Árendás, T., Bónis, P., Csathó, P., Molnár, D., Berzsenyi, Z. (2010): Fertiliser responses of maize and winter wheat as a function of year and forecrop. *Acta Agron. Hung.*, **58**, 109–114.

Berzsenyi, Z. (1996): A N-műtrágyázás hatásának vizsgálata a kukorica (Zea mays L.) növekedésére Hunt–Parsons modellel. (Studies on the effect of N fertilisation on maize (*Zea mays* L.) growth using the Hunt–Parsons model.) *Növénytermelés*, **45**, 35–52.

Berzsenyi Z. (2000): Növekedésanalízis a növénytermesztésben. (Growth analysis in crop production). (Review). *Növénytermelés*, **49**, 389–403.

Berzsenyi Z. (2002): A növekedésanalízis funkcionális módszere. (Functional method of growth analysis). (Review). *Növénytermelés*, **51**, 449–467.

Causton, D.R., Venus, J. C. (1981): *The Biometry of Plant Growth.* Edward Arnold, London.

Evans, G. C. (1972): *The Quantitative Analysis of Plant Growth.* Blackwell Scientific Publications, Oxford.

Hunt, R. (1982): *Plant Growth Curves: The Functional Approach to Plant Growth Analysis.* Edward Arnold, London.

Hunt, R., Parsons, I. T. (1974): A computer program for deriving growth-functions in plant growth analysis. *J. Appl. Ecol.*, **11**, 297–307.

Micskei, G., Jócsák I., Berzsenyi Z. (2010): Studies on the effect of farmyard manure and mineral fertiliser on the growth of maize (*Zea mays* L.) in a long-term experiment. I. Using the classical form of plant growth analysis. *Acta Agron. Hung.*, **59**, 227–238.

Sugár E., Berzsenyi Z. (2009): Őszi búzafajták növekedésdinamikája és termésprodukciója eltérő N-tápelemszinteken. (Growth dynamics and yield of winter wheat varieties grown at diverse nitrogen levels.) *Tartamkísérletek jelentősége a növénytermesztés fejlesztésében.* Jubileumi tudományos konferencia, Martonvásár. pp. 159–164.

Sugár E., Berzsenyi Z. (2010): Growth dynamics and yield of winter wheat varieties grown at diverse nitrogen levels. *Acta Agron. Hung.*, **58**, 121–126.

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EVALUATION OF FORAGE QUALITY OF *Lathyrus* L. SPECIES BASED ON HISTOLOGICAL CHARACTERISTICS

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One of the most important characteristics that determines the quality of forages is their digestibility. Certain structural characteristics of the vegetative organs, such as a high percentage of cells with lignified walls, might limit digestibility. Leaf and stem histological characteristics related to digestibility were examined for nine wild *Lathyrus* species closely related to cultivated species. The recommended characteristics for plants that could be used as forage on their own are: a small number of stem vascular bundles, a relatively small proportion of stem sclerenchyma and sclerenchymatous parenchyma tissue, thin leaflet cuticle, a small number of leaf vascular bundles, and large mesophyll and epidermal cells. According to the results of the analyses, species with favourable histological characteristics were *L. palustris, L. sphaericus* and *L. aphaca,* while the species with the highest proportion of thick-walled cells, unsuitable for use as forage, were *L. pratensis*, *L. niger* and *L. tuberosus*.

Key words: digestibility, histology, *Lathyrus,* thick-walled cells

Introduction

Lathyrus species have been cultivated for centuries as protein-rich legumes for human food and as grain, forage and green manure crops (Miyan and Bellotti, 1998). Besides the utilization of seeds, *Lathyrus* plants can be cut and fed green, or the standing crop can be pastured. Among the species of this genus, *L. sativus* and *L. cicera* are of the highest agricultural importance (Lopez Bellido, 1994; Abreu and Bruno-Soares, 1998; Chtourou-Ghorbel et al., 2001; White et al., 2002). The seed is the part mainly used, although the species are also grown as green fodder or hay. Both cultivated and wild-growing *Lathyrus* species are often utilized by grazing animals in their native habitat. One of the most important characteristics that determines the quality of plants used as forage is their digestibility. It is known that dry matter digestibility depends on

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the fibre content in plant organs and decreases with plant maturity, mainly because the fibre content increases (Akin and Robinson, 1982; Julier and Huyghe, 1997; Guines et al., 2003). During plant maturation, the digestibility of legume stems declines more rapidly than that of leaf blades, because they have greater fibre content (Buxton and Redfearn, 1997). The very high concentration of lignin in the lignified cell walls of legumes is responsible for limiting digestibility and making their degradation difficult (Wilman and Rezvani Moghaddam, 1998). A high proportion and large number of thick-walled cells imply low digestibility. However, lignified tissues are necessary to provide mechanical support and mechanical resistance to diseases and other biotic and abiotic stresses, which limits the possibilty of improving forage quality through reduced lignin concentration (Buxton and Redfearn, 1997).

Although it is very important to understand the factors that limit forage quality, very little research has been carried out on the relationship of plant structure to digestibility in legumes. Guines et al. (2003) proved that the histological structure of lucerne stems affected the digestibility of this forage. On the basis of histological parameters, the forage quality of wild-growing *Trifolium* species was evaluated by Krstic et al. (2008). A reduction in the proportion of lignified xylem, sclerenchyma and sclerenchymatous interfascicular parenchyma, particularly in stems and peduncles, was suggested as a basic selection criterion in breeding *Trifolium* species for improved digestibility. However, no data on this subject were recorded for *Lathyrus* species. Therefore, the aim of the present research was to determine the histological structure of aboveground vegetative organs of nine wild *Lathyrus* species closely related to cultivated species, in order to identify the histological characteristics significant for digestibility, to assess the variability in the proportion of different tissues composed of thick-walled cells in leaflets and stems, to evaluate the potential of wild-growing species to be used as forage and to contribute information which could be useful for pasture management.

Materials and methods

Plant material was collected at the beginning of flowering from wild-growing populations at Fruska Gora (North Serbia). Plants were collected at the same time and from the same locality, so the effect of environmental factors on differences between the species in the structure of the plant organs was minimized. The species examined were: *L. aphaca* L., *L. hirsutus* L., *L. latifolius* L., *L. niger* (L.) Bernh., *L. palustris* L., *L. pratensis* L., *L. sphaericus* Retz., *L. tuberosus* L. and *L. vernus* (L.) Bernh. The plants were identified at the Department of Biology and Ecology, University of Novi Sad. Voucher specimens were deposited in the Herbarium of the Department of Biology and Ecology, University of Novi Sad (BUNS). Thirty plants of each species, sampled from the same population, were used for histological studies. Segments of stems and leaves from the middle part of the plants were separated and fixed in 50% ethanol. Stipules were analysed for *L. aphaca*. Cross-sections of stems and median lateral leaflets were made using a Leica CM 1850 cryostat at a temperature of –18°C to –20°C, at cutting intervals of 25 μm. The sections were stained for lignin using the acid phloroglucinol test (Jones et al., 2003)

Light microscopy observations and measurements were made using an Image Analyzing System Motic 2000. For the stem, the cross-section areas of the stem, xylem, sclerenchyma and sclerenchymatous parenchyma were measured and the number of vascular bundles was determined. For the leaflets, the mesophyll and cuticle thickness, the cross-section areas of the epidermal and mesophyll cells and the cross-section areas of the leaflets and vascular bundles, together with the sclerenchyma tissue, were measured. Relative proportions were calculated for each tissue and expressed as a ratio compared to the whole cross-section area of each organ or plant part. The data were statistically processed by analysis of variance, and means, standard errors, coefficients of variation and correlation coefficients were calculated using STATISTICA for Windows version 7.0 (StatSoft Inc., 2004). The significance of differences in measured parameters between the species was determined using Duncan's test ($p \leq 0.05$). The general structure of sample variability was established using Principal Component Analysis (PCA), based on the correlation matrix. Multivariate Discriminant Function Analysis (MDA) was done in order to check the hypothesis that the analysed sample was composed of groups which were differentiated from each other.

Results

The stem cross-sections of *Lathyrus* species are round to oval in shape, with two prominent ribs. All the stems have two wing-like expansions of various length. In most of the species, these contain only one vascular bundle each, while there are 4–6 bundles in *L. palustris*, 8–10 in *L. hirsutus* and 9–13 in *L. latifolius*. All of the species have a single-layered epidermis, covered with a cuticle. Subepidermally, collenchyma tissue occurs in the two main ribs and at the top of the expansions. Some species, such as *L. pratensis, L. vernus* and *L. aphaca*, have lacunae in the primary cortex, under the epidermis. The cortex is rather thin, usually composed of 2–3 layers of parenchyma cells containing chloroplasts. A diverse number of vascular bundles, ranging from 6–8 in *L. palustris* to 20–24 in *L. hirsutus,* are present at the periphery of the central cylinder. The vascular bundles in the ribs are prominent, while others, including those in the wing-like expansions (cortical bundles), are much smaller. Sclerenchyma tissue occurs above the phloem of each vascular bundle, with narrower fibres and thicker cell walls towards the periphery. The parenchyma sheath cells, above the sclerenchyma, contain solitary crystals. These are very rare in *L. sphaericus* and *L. aphaca* stems. The interfascicular parenchyma cells have lignified cell walls and form sclerenchymatous parenchyma. Other parenchyma cells of the central cylinder are thin-walled and are larger towards the stem centre. The largest central cells may rupture and form a cavity. In some species, such as *L. pratensis, L. vernus, L. hirsutus, L. sphaericus* and *L. aphaca*, the parenchyma cells under the vascular bundles contain starch grains.

Analyses of the stem anatomical structure showed that the tissues composed of thick-walled cells were mechanical tissue (sclerenchyma), vascular tissue (xylem) and sclerenchymatous parenchyma tissue. Because of the lignin contained in the cell walls, these tissues are digested very slowly, or are sometimes completely indigestible (Buxton and Redfearn, 1997). It should be noted that the cell walls of collenchyma cells are unevenly thickened, but not lignified, and therefore their digestibility, according to Buxton and Redfearn (1997), is moderate to high. In most of the species the epidermis is made up of

cells where only the outer wall is thickened, so these cells were not assigned as thick-walled in this study. Phloem tissue also contains thick-walled fibres, but as the cell walls do not often lignify and other phloem cells have thin walls, this tissue was not classified as having thick-walled cells.

The species with the highest proportion of thick-walled cells in the stem were *L. tuberosus* and *L. niger* (Table 1). Among the examined species, *L. tuberosus* had significantly the highest percentage of sclerenchymatous parenchyma (the differences were not significant except compared to *L. hirsutus* and *L. latifolius*). *L. niger* had significantly the highest percentage of sclerenchyma and xylem tissue. The species with the lowest percentage of thick-walled cells were *L. palustris, L. sphaericus* and *L. aphaca. L. palustris* had significantly the lowest percentage of sclerenchymatous parenchyma. The percentage of xylem was significantly the lowest in *L. palustris, L. sphaericus* and *L. latifolius*. *L. niger* showed the highest variation for the parameters analysed, while high coefficients of variation were also recorded for *L. vernus*. It was interesting to note that species containing the lowest percentage of thick-walled cells (*L. aphaca, L. sphaericus* and *L. palustris*) showed little variation in the percentage of sclerenchymatous parenchyma.

Correlations were calculated between the stem cross-section area and the percentages of tissues composed of thick-walled cells, but these were not statistically significant, except for the sclerenchyma tissue of *L. palustris* (0.983) and the xylem of *L. hirsutus* (0.987). Correlations were also detected between the proportions of the measured tissues, but these were not significant for any of the species examined.

Table 1
Percentage of tissues composed of thick-walled cells in stem cross-sections
(mean ± standard error and coefficient of variation %)

Species	Sclerenchyma (%)	Xylem (%)	Sclerenchymatous parenchyma (%)	Total percentage of thick-walled cells (%)	No. of cylinder vascular bundles
L. aphaca	7.4±0.5 cd*	15.6±0.5 c	16.5±0.5 bc	39.5±1.0 c	11±0 bc
	15.8	6.9	6.4	5.7	9.1
L. hirsutus	6.0±0.2 d	17.2±0.5 bc	20.4±0.9 ab	43.6±0.9 bc	22±1 a
	8.3	6.6	9.7	4.8	7.3
L. latifolius	7.3±0.4 cd	11.1±0.9 d	24.9±1.9 a	43.3±1.3 bc	20±2 a
	11.6	17.2	16.7	6.8	24.3
L. niger	14.2±1.2 a	21.8±2.8 a	19.6±2.9 b	55.6±3.0 a	20±3 a
	18.3	28.7	33.3	12.1	38.8
L. palustris	6.9±0.6 cd	9.5±0.9 d	12.8±0.4 c	29.3±1.2 d	7±0 c
	17.0	19.6	5.5	9.0	14.7
L. pratensis	8.6±0.5 c	19.9±1.6 ab	18.4±2.3 b	46.9±1.0 b	13±1 b
	12.1	17.4	28.0	4.9	9.7
L. sphaericus	10.3±0.3 bc	11.1±0.5 d	18.2±0.5 b	39.6±0.6 c	10±1 bc
	5.8	9.5	5.5	3.6	14.0
L. tuberosus	10.8±0.3 b	19.3±1.2 abc	25.3±1.8 a	55.4±1.4 a	10±1 bc
	5.7	13.4	16.1	5.8	17.6
L. vernus	14.2±0.9 a`	12.1±1.3 cd	19.1±1.7 b	45.4±2.6 b	13±1 b
	14.8	24.7	19.5	12.8	14.4

* Differences between samples designated with the same letter were not significant at $p<0.05$.

Another anatomical characteristic important for plant digestibility is the number of stem vascular bundles. As these are composed of thick-walled xylem elements, phloem (which also contains thick-walled fibres) and the sclerenchyma next to the vascular tissue, the most favourable characteristic with respect to digestibility would be a small number of vascular bundles. Among the species examined, the lowest number of vascular bundles in the central cylinder was observed for those with the lowest percentage of thick-walled cells: *L. palustris, L. sphaericus* and *L. aphaca,* as well as *L. tuberosus* (Table 1). *L. palustris* also had 4–6 cortical bundles in each wing-like expansion, but these bundles were very small, and thus did not contribute much to the total percentage of thick-walled cells.

The leaflets of all *Lathyrus* species have a dorsiventral structure. The epidermis is formed of one layer of cells, covered with a cuticle. In most of the species, the palisade tissue is composed of one layer of narrow, elongated, cylindrical cells, while *L. pratensis* and *L. vernus* have short palisade tissue cells, more cubic in shape. The spongy tissue cells are of irregular shape and usually arranged in three to four layers. *L. tuberosus* and *L. sphaericus* have 4–5 and *L. latifolius* 5–6 layers of spongy tissue cells. In these three species, the spongy layer under the abaxial epidermis is formed of somewhat larger, elongated cells, which resemble palisade tissue cells. Small collateral vascular bundles are present in the mesophyll and they are surrounded by parenchyma sheath cells containing small solitary crystals. These crystals are very rare in *L. palustris* and very numerous in *L. hirsutus* leaflets. The main vein is convex abaxially. It contains one large vascular bundle with well-developed sclerenchyma along the phloem portion. The bundle sheath cells contain crystals. The groups of collenchyma tissue are present only in the main vein, abaxially. The anatomical structure of the stipules of *L. aphaca* resembles the structure of the leaflets in other *Lathyrus* species. The assimilatory parenchyma is differentiated on one-layered palisade tissue and spongy tissue made up of 2–3 layers of cells. No collenchyma tissue is present.

Analyses showed that the leaflet tissues composed of thick-walled cells were vascular and sclerenchyma tissue, while the photosynthetic tissue was classified as tissue composed of thin-walled cells. Cuticle thickness is another important leaflet characteristic, because a thick cuticle limits the access of rumen bacteria to other leaflet tissues. The cuticle thickness on both leaf surfaces was similar for all the species examined (Table 2). The *L. aphaca* stipules had a very thin cuticle, and the difference was significant compared to the other species.

A lower proportion of vascular and mechanical tissue is a favourable selection criterion for the improvement of leaf digestibility. The species with the highest proportion of leaf tissues composed of thick-walled cells were *L. latifolius, L. pratensis* and *L. hirsutus*, while the species with the lowest proportion were *L. aphaca* and *L. vernus* (Table 2). The highest values of coefficients of variation were calculated for *L. latifolius,* while *L. hirsutus* and *L.*

palustris showed little variability in the percentage of thick-walled cells. Correlations between the leaflet cross-section area and the percentage of thick-walled cells were not significant for any of the species analysed, which meant that leaflet size did not influence the proportion of tissues made up of thick-walled cells.

The largest mesophyll cells were recorded for *L. latifolius* and *L. palustris*, and the differences were statistically significant compared to the other species at $p<0.05$ (Table 2). *L. pratensis, L. vernus* and *L. aphaca* had significantly the smallest palisade cells and *L. pratensis* the smallest spongy cells. The epidermal cells were significantly the largest for *L. latifolius,* and significantly the smallest for *L. vernus* and *L. aphaca.* Among the species examined, *L. latifolius* could be singled out as the one with the thickest mesophyll and the largest thin-walled cells, while *L. pratensis, L. vernus* and *L. aphaca* were the species with the thinnest mesophyll and the smallest thin-walled cells.

Table 2

Percentage of tissues composed of thick-walled cells in leaflet cross-sections and descriptive statistics of leaflet anatomical characteristics (mean ± standard error and coefficient of variation %)

Species	% of vascular + sclerenchyma tissue	Mesophyll thickness (μm)	Cuticle thickness (μm)		Cross-section area (μm^2)			
			ade*	abe	ade cells	abe cells	palisade cells	spongy cells
L. aphaca	7.4±0.9c	92.4±3.8d**	1.8±0.1b	1.8±0.1b	234±15f	231±18c	640±32fg	444±27cd
(stipules)	20.3	12.9	11.4	16.8	31.1	38.5	25.2	29.9
L. hirsutus	12.8±0.4ab	137.9±5.1bc	1.9±0.1ab	2.0±0.1ab	344±21ef	499±29b	935±48de	419±24cd
	7.0	10.5	15.5	10.1	27.7	25.6	22.8	25.2
L. latifolius	15.8±2.6a	207.1±8.4a	2.1±0.1a	2.2±0.1a	866±57a	706±33a	1408±47b	714±33a
	36.7	12.8	14.4	13.5	33.0	23.7	16.8	23.2
L. niger	8.4±0.5c	137.3±6.1bc	2.1±0.1a	2.0±0.1ab	718±49b	408±33b	1164±76c	422±21cd
	11.9	14.0	9.6	9.6	33.7	40.2	32.7	25.1
L. palustris	9.3±0.3c	154.4±8.3b	2.0±0.1a	2.1±0.1a	517±38cd	658±62a	1616±135a	744±45a
	7.5	15.2	19.7	9.4	33.0	42.2	37.3	26.7
L. pratensis	13.4±0.9ab	74.8±6.2d	2.2±0.1a	2.2±0.1a	444±30de	463±47b	504±36g	206±12e
	13.4	23.3	9.3	18.4	29.8	45.2	31.5	26.0
L. sphaericus	9.2±0.5c	131.7±9.1c	1.9±0.1ab	2.0±0.1ab	592±62c	470±37b	809±46ef	580±48b
	12.0	21.9	16.0	15.0	52.2	39.2	28.7	41.1
L. tuberosus	10.7±0.8bc	158.2±7.9b	2.1±0.1a	2.0±0.1a	594±33c	513±44b	1114±82cd	385±24d
	15.9	15.8	14.2	18.2	27.6	42.9	36.9	30.7
L. vernus	8.0±0.6c	87.5±3.0d	1.9±0.1ab	2.0±0.1ab	293±24f	226±23c	621±35fg	506±30bc
	15.0	11.0	21.1	10.1	40.5	51.0	28.0	29.7

* ade: adaxial epidermis, abe: abaxial epidermis; ** Differences between samples designated with the same letter were not significant at $p<0.05$.

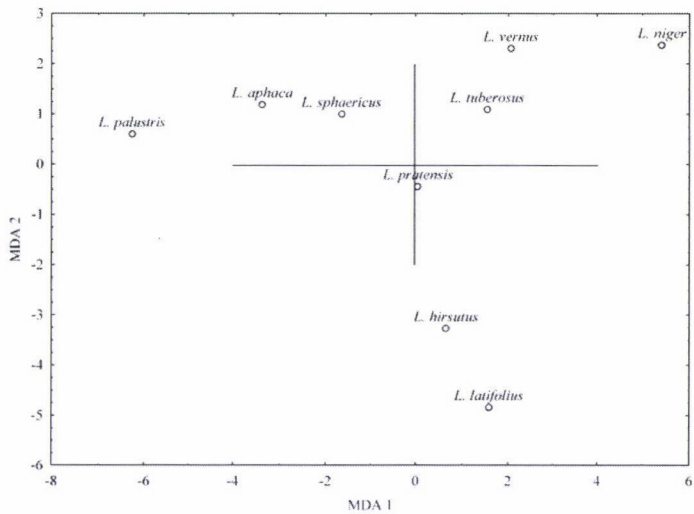

Fig. 1. Results of Multivariate Discriminant Analysis (MDA), projection of the first two factors

The relationship between stem digestibility and anatomy was previously reported by Guines et al. (2003) and Jung and Lamb (2003) for lucerne stems. In the present study, variability in the histological characteristics connected with digestibility and fibre content was shown for *Lathyrus* species. On the basis of the results of histological analyses, the recommended characteristics for plants suitable for animal use are: a small number of stem vascular bundles (therefore, a smaller proportion of xylem), a relatively small proportion of stem sclerenchyma and sclerenchymatous parenchyma tissue, thin leaflet cuticle, a small number of leaflet vascular bundles and large mesophyll and epidermal cells.

Correlations between stem size (stem cross-section area) and the percentage of xylem, sclerenchyma and sclerenchymatous parenchyma tissue were not significant, and nor were correlations between leaflet cross-section area and the percentage of vascular and sclerenchyma tissues. A positive correlation between plant size and fibre concentration could be expected for plants at different stages of maturity (Akin and Robinson, 1982; Buxton and Redfearn, 1997; Guines et al., 2003). For *Lathyrus* plants at the same stage of maturity, such correlations were not detected, which is in agreement with the results of Krstic et al. (2008) for *Trifolium* species.

According to the results of histological analyses, species with desirable characteristics, suitable to be used as forage, could be singled out. These were *L. palustris, L. sphaericus* and *L. aphaca.* Besides having the lowest proportion of tissues composed of thick-walled cells, *L. palustris* also had a small number of stem vascular bundles, relatively large mesophyll cells and a small number of leaf crystals, which are also unsuitable for digestion. The characteristics of *L. aphaca*, such as a low percentage of thick-walled cells, a small number of stem vascular bundles, thin cuticle on the stipules and the absence of collenchyma tissue in the stipules, were also favourable for high digestibility.

Considering the results of PCA analysis, it could be seen that variation exists especially in the size of mesophyll and epidermal cells. The characters examined generally had low variability. Three groups of characters explained 60.78% of the total variation (Table 3). The first principal component explained 28.48% of the variation. It was defined by the cross-section areas of palisade and epidermal cells. The second and third principal components explained 20.01% and 12.29% of the variation, respectively. The percentage of leaf vascular and sclerenchyma tissue, cuticle thickness, the percentage of stem sclerenchymatous parenchyma and the number of stem vascular bundles showed the lowest variability.

As also suggested by the literature, the percentage of tissues composed of thick-walled cells and the number of stem vascular bundles proved to be the most important for forage quality, so these characters were further treated using Multivariate Discriminant Analysis (MDA). MDA was performed in order to check the differentiation of the species examined. The results showed that two groups of species could be clearly separated, according to the first corresponding axis (Fig. 1). The first group, on the left side, was composed of *L. palustris, L. sphaericus* and *L. aphaca*, the species with the lowest proportion of tissues composed of thick-walled cells in the stems and a low proportion in the leaves. The second group included species with the highest proportions of thick-walled cells, *L. niger, L. tuberosus* and *L. vernus.*

Discussion

One of the most important objectives in legume forage breeding programmes aimed at improving digestibility is a reduction in the fibre content in stems and leaves. The amount of lignin that can be reduced in forages is limited, because it provides the plants with resistance to diseases, insects and cold temperatures (Buxton and Redfearn, 1997). A certain amount of sclerenchyma tissue is necessary in the leaves to provide leaf strength, optimum position and the ability to withstand the effects of various environmental factors, such as drought or temperature extremes (Rezvani Moghaddam and Wilman, 1998).

Table 3

Principal component analysis (PCA) on the measured parameters. Factor coordinates of the variables, based on correlations (marked loadings are >0.700) and cumulative percentages of the vectors

		Factor 1	Factor 2	Factor 3
Leaf	% Vascular+sclerenchyma tissue	–0.494806	–0.428151	0.526639
	Cross-section area palisade cells	–0.814366*	0.261720	–0.268625
	Cross-section area spongy cells	–0.603709	0.649325	–0.114741
	Cross-section area ade cells	–0.722759*	–0.288499	–0.246225
	Cross-section area abe cells	–0.860937*	0.035088	0.165847
	Cuticle thickness ade	–0.440907	–0.425660	–0.225793
	Cuticle thickness abe	–0.428237	–0.171369	–0.387317
Stem	% Xylem	0.241453	–0.709147*	–0.175196
	% Sclerenchyma	0.329329	–0.179003	–0.788265*
	% Scler. parenchyma	–0.181990	–0.498240	–0.022506
	Number of v. bundles	–0.139279	–0.670729	0.217085
Cumulative percentages of the vectors		28.48	48.50	60.78

The species with the highest proportion of thick-walled cells were *L. pratensis* (which also had a thin mesophyll and relatively small mesophyll cells), *L. niger* (a species with a relatively large number of stem vascular bundles) and *L. tuberosus*. On the basis of their histological characteristics, those species were not recommended for animal consumption. Further morphological, biochemical and molecular investigations are needed to obtain a more complete view of wild-growing *Lathyrus* species as possible forage plants.

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References

Abreu, J. M. F., Bruno-Soares, A. M. (1998): Chemical composition, organic matter digestibility and gas production of nine legume grains. *Anim. Feed Sci. Tech.*, **70**, 49–57.

Akin, D. E., Robinson, E. L. (1982): Structure of leaves and stems of arrowleaf and crimson clovers as related to *in vitro* digestibility. *Crop Sci.*, **22**, 24–29.

Buxton, D. R., Redfearn, D. D. (1997): Plant limitations to fiber digestion and utilization. *J. Nutr.*, **127**, 814–818.

Chtourou-Ghorbel, N., Lauga, B., Combes, D., Marrakchi, M. (2001): Comparative genetic diversity studies in the genus *Lathyrus* using RFLP and RAPD markers. *Lathyrus Lathyrism Newsletter*, **2**, 62–68.

Guines, F., Julier, B., Ecalle, C., Huyghe, C. (2003): Among and within-cultivar variability for histological traits of lucerne (*Medicago sativa* L.) stem. *Euphytica*, **130**, 293–301.

Jones, A., Reed, R., Weyers, J. (2003): *Practical Skills in Biology*. Pearson Education Ltd., UK.

Julier, B., Huyghe, C. (1997): Effect of growth and genotype on alfalfa digestibility in a multilocal trial. *Agronomie*, **17**, 481–489.

Jung, H. J. G., Lamb, J. F. S. (2003): Identification of lucerne stem cell wall traits related to *in vitro* neutral detergent fiber digestibility. *Anim Feed Sci. Tech.*, **110**, 17–29.

Krstic, L., Merkulov, L., Lukovic, J., Boza, P. (2008): Histological components of *Trifolium* L. species related to digestive quality of forage. *Euphytica*, **160**, 277–286.

Lopez Bellido, L. (1994): Grain legumes for animal feed. pp. 273–288. In: Hernando Bermejo, J. E., Leon, J. (eds.), *Neglected Crops: 1492 from a Different Perspective*. Plant Production and Protection Series No. 26, FAO, Rome.

Miyan, M. S., Bellotti, W. D. (1998): Agronomy of *Lathyrus* species in South Australia. *Proceedings of the 9th Australian Agronomy Conference*, Wagga Wagga.

Rezvani Moghaddam, P. R., Wilman, D. (1998): Cell wall thickness and cell dimensions in plant parts of eight forage species. *J. Agric. Sci.*, **131**, 59–67.

StatSoft, Inc. (2004): STATISTICA (data analysis software system), version 7. www.statsoft.com

White, C. L., Hanbury, C. D., Young, P., Phillips, N., Wiese, S. C., Milton, J. B., Davidson, R. H., Siddique, K. H. M., Harris, D. (2002): The nutritional value of *Lathyrus cicera* and *Lupinus angustifolius* grain for sheep. *Anim. Feed Sci. Tech.*, **99**, 45–64.

Wilman, D., Rezvani Moghaddam, P. R. (1998): Volume, surface area and cellular composition of chewed particles of plant parts of eight forage species and estimated degradation of cell wall. *J. Agric. Sci.*, **131**, 69–77.

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GENETIC CORRELATION AND COHERITABILITY BETWEEN F_1 AND F_2 GENERATIONS FOR QUANTITATIVE TRAITS IN SOME CROSSES OF GREEN GRAM (*Vigna radiata* L. Wilczek)

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Quantitative data were collected and analysed on 10 metric characters from F_1s and F_2s of sixteen green gram (*Vigna radiata* L. Wilczek) hybrids, developed from ten genetically diverse parents, to obtain information on variability, heritability, coheritability, and genotypic and phenotypic correlations. The increased genetic variability of F_2 over F_1 was observed for plant height, number of primary branches, pods/cluster, pods/plant, pod length and harvest index, suggesting the greater segregation and recombination of genes governing these characters. The high broad-sense heritability coupled with high genetic advance for plant height, days to 50% flowering and pod length in both F_1s and F_2s indicated the predominant role of additive gene action in their expression. The genotypic and phenotypic correlations between F_1 and F_2 were significant and positive for seven characters. The coheritability estimates between F_1 and F_2 had values of over 0.25 and were positive for six characters, but not for seed yield/plant, days to 50% flowering, clusters/plant or pods/plant. Within the F_1 and F_2 generations, seed yield/plant showed significant positive genotypic and phenotypic correlations with eight characters. The seed yield in green gram could be easily enhanced by practising selection on plant height, number of primary branches, pods/cluster, pod length and harvest index.

Key words: green gram, F_1, F_2, correlation, coheritability, yield, yield components

Introduction

Green gram (*Vigna radiata* L. Wilczek), grown mostly by resource-poor farmers in South and South-East Asia (Gupta et al., 2004) and mainly as a source of vegetable protein due to its high protein content of about 23% (Naik and Kole, 2002), is often preferred to other pulse crops for its easy digestibility in the stomach. The average productivity of green gram is much lower than that of rice all over the globe and hence green gram is priced at almost four times the market price of rice in India. The high price of all the pulse crops has indirectly reduced the daily intake of legumes in Indian diets over the last decade. This calls for the urgent attention of the pulse breeders and policy makers of India

and other developing nations to raise the grain yield of all pulse crops, including green gram, in order to check the inordinate price rise and to increase the per capita availability of pulses in the diet.

To increase the grain yield, it is essential to evolve a high yielding, stable, pure-line variety of green gram, an autogamous species, by selection from the segregating generations of superior crosses. The potential of the F_1 hybrids is generally reflected in the succeeding segregating generations. Yonezawa and Yamagata (1981) showed that the potential of a cross can be determined in the F_2/F_3 generation. Grain yield is a complexly inherited character, influenced by several component characters and by the environment. Direct selection for yield is often not effective. It is therefore necessary to identify effective component characters that are highly correlated to grain yield genetically on the basis of correlation studies in order to formulate a successful breeding programme. Green gram shows a high G–E interaction, which causes variation in the mean values of genotypes for quantitative characters with changes in the growing season and fertility levels (Naidu and Satyanarayana, 1991).

The present study was undertaken to estimate the variability, genotypic and phenotypic correlations, and coheritability for quantitative characters between the F_1 and F_2 generations of green gram crosses and also between the seed yield and its components within each generation.

Materials and methods

Sixteen promising F_1 hybrids of green gram, involving ten genetically diverse parents (Table 1), were grown in the summer season in a randomized block design with three replications in the Regional Agricultural Research Station, Nagaon. Each cross was raised in 5 rows 5 metres in length with a crop geometry of 30 cm × 10 cm and a fertilizer dose of N : P : K = 15 : 35 : 0 kg/ha. Data were recorded for ten quantitative characters (seed yield and nine component characters) from 20 randomly selected plants of each cross in each replication.

The F_2 generations of each cross were also grown in the same experimental design as the F_1 in the subsequent wet season in larger plots of 10 rows 10 metres in length with the same crop geometry and same fertilizer dose. Data were recorded on ten quantitative characters from 100 randomly selected plants of each cross in each replication.

Table 1
Crosses tested in the experiment

No.	Cross	No.	Cross
1.	SG1/T44	9.	K851/T44
2.	ML5/K851	10.	SG1/AAU34
3.	WGG62/PantM2	11.	SG1/K851
4.	SG1/WGG62	12.	SG1/PantM2
5.	PantM2/PDM90237	13.	PDM91243/WGG62
6.	SG1/MUM5	14.	T44/WGG62
7.	SG1/ML5	15.	K851/WGG62
8.	PDM91243/PDM90237	16.	AAU34/T44

Biometrical analysis

The mean values of the characters were used for analysis of variance and covariance as described by Singh and Chaudhary (1985). The variability parameters GCV and PCV were calculated according to Burton (1952) and the broad-sense heritability and expected genetic advance at 5% selection intensity according to Johnson et al. (1955). Genotypic and phenotypic correlation coefficients between any two characters in each generation and also between two generations (F_1 & F_2) for the same character were estimated as reported by Panse and Sukhatme (1957) and were tested using the t-test for significance. Coheritability between two characters or two generations was estimated according to Nei (1960).

Results and discussion

Analysis of variance

Analysis of variance for all the quantitative characters in 16 green gram crosses revealed that the crosses differed significantly at $p = 0.01$ (values not shown), indicating the presence of ample genetic variation among the crosses for all the characters. Similar results were reported by Kalita and Hazarika (1997) in two green gram crosses.

Table 2

Magnitude of genetic parameters of different quantitative characters in F_1 and F_2 generations of 16 green gram crosses

Character	Filial generation	Mean value	GCV (%)	PCV (%)	Broad-sense heritability (%)	Genetic advance as % of mean
Plant height (cm)	F_1	72.47	16.49	20.10	67.79	27.94
	F_2	65.14	18.04	19.29	87.36	34.72
No. of primary branches	F_1	3.39	13.28	30.08	27.35	16.95
	F_2	2.22	24.32	50.06	33.62	24.55
Days to 50% flowering	F1	65.00	3.84	4.01	92.63	7.65
	F_2	52.70	2.50	3.34	56.77	3.90
Clusters/plant	F_1	23.04	24.00	37.10	41.73	31.90
	F_2	10.41	11.23	26.89	17.63	9.82
Pods/cluster	F_1	5.50	8.33	20.16	27.07	7.09
	F_2	4.83	17.18	29.19	34.50	20.74
Pods/plant	F_1	66.12	17.60	44.43	25.71	14.38
	F_2	28.10	18.14	34.23	28.17	19.86
Pod length (cm)	F_1	7.32	9.69	12.29	62.19	15.75
	F_2	7.14	11.20	13.31	71.42	19.57
Seeds/pod	F_1	12.50	7.44	13.04	32.83	8.82
	F_2	11.70	4.64	8.80	27.64	12.63
Harvest index	F_1	0.29	15.16	27.00	53.14	14.19
	F_2	0.27	16.12	24.04	47.17	12.12
Seed yield/plant (g)	F_1	6.55	25.64	42.90	36.21	32.00
	F_2	6.44	19.09	34.62	30.72	34.62

Variability

The F_1 mean value for each character was higher than that of F_2 (Table 2). In general the F_2 GCV (genotypic coefficient of variability) estimates were only higher than the F_1 GCV estimates, as expected, for six characters, namely plant height, number of primary branches, pods/cluster, pods/plant, pod length and harvest index. In faba bean, higher F_4 mean values over F_2 were observed for branches/plant, pods/plant, seeds/plant and seed yield/plant (Ahmed et al., 2008). Like GCV estimates, the F_2 PCV (phenotypic coefficient of variability) estimates showed an almost similar trend in comparison to the F_1 PCV estimates. The increase in F_2 GCV and PCV estimates compared to those of F_1 for six characters could be due to greater segregation and recombination of the polygenes governing these characters, coupled with an environmental effect through the G × E interaction. On the other hand, the lower estimates of F_2 GCV and PCV for four characters in comparison to those of F_1 could be due to environmental effects, resulting in narrowing down the variability. Variation in the mean values of characters was observed as a function of growing seasons and fertility levels (Del Rio et al., 1997; Ramana and Singh, 1987).

Heritability and genetic advance

The higher estimates of F_2 broad-sense heritability and expected genetic advance (Table 2) compared to F_1 for several characters (plant height, number of primary branches, pods/cluster, pods/plant, pod length and harvest index) revealed that selection would be more effective in F_2 than in the F_1 generation. Rehman et al. (2009) also observed higher heritability for grain yield in F_2 compared to F_1 in green gram. The reverse trend was observed for the remaining characters. In general, both the generations showed high heritability with high genetic advance for plant height, days to 50% flowering and pod length, suggesting the predominant role of additive gene action in the expression of these characters. Similar results were reported by Rehman et al. (2009) for the grain yield in F_2 and the harvest index in F_1, by Ramana (1985) for plant height and by Medhi et al. (1980) for pod length. Other characters showed moderate heritability estimates. Similar results were reported by Tomar et al. (1972) for branches/plant, by Singh and Malhotra (1970) for clusters/plant, by Parida and Singh (1984) for pods/plant and by Empig et al. (1970) for seeds/pod and seed yield/plant.

Correlation and coheritability

Significant positive genotypic and phenotypic correlations between the F_1 and F_2 generations (Table 3) were observed for six characters (plant height, number of primary branches, pods/cluster, pod length, seeds/pod, harvest index) and only a genotypic correlation for yield/plant. This revealed that selection in the F_1 generation would be more effective for these characters. The coheritability estimates between the F_1 and F_2 generations were high (greater than 0.25 and positive) for the same characters, except seed yield/plant and days to 50% flowering.

Table 3
Correlation coefficients between F_1 and F_2 generations for each quantitative character from a set of 16 green gram crosses

Character[+]	Genotypic correlation coefficient	Phenotypic correlation coefficient	Coheritability between F_1 & F_2 generations
Plant height (cm)	0.71**	0.61**	0.53
No. of primary branches	0.48**	0.62**	0.31
Days to 50% flowering	0.21	0.16	0.15
Clusters/plant	–0.23	0.02	–0.05
Pods/cluster	0.80**	0.23*	0.29
Pods/plant	–0.69**	–0.16	–0.14
Pod length (cm)	0.95**	0.60**	0.71
Seeds/pod	0.79**	0.36**	0.37
Harvest index	0.72**	0.46**	0.39
Seed yield/plant (g)	0.42**	0.13	0.13

[+]in which correlation was estimated between F_1 & F_2; *, ** Significant at the $p = 0.05$ and $p = 0.01$ level, respectively

In both F_1 and F_2, the seed yield/plant showed a significant positive genotypic correlation with all the characters except days to 50% flowering and seeds/pod (Table 4). A similar trend was observed for the phenotypic correlation. As in the present study, a significant positive correlation between seed yield/plant and other characters was reported by several workers, namely branches/plant (Singh et al., 1975), clusters/plant (Yohe and Poehlman, 1975) and pods/plant and pod length (Singh and Sharma, 1981). A negative correlation was observed by Singh et al. (1968) between seed yield and days to 50% flowering. In the F_2 of some wide and varietal crosses in green gram, Parida and Singh (1984) observed a positive correlation between seed yield/plant and both pods/plant and seeds/plant. The F_1 coheritability estimate for seed yield/plant was positive and greater than 0.25 with respect to plant height and clusters/plant, and in the F_2 in terms of days to 50% flowering and pods/cluster.

The results of the present study revealed that the F_2s showed greater variability than the F_1s for most quantitative traits, thus following the Mendelian inheritance pattern. Significant positive genotypic and phenotypic correlations were observed between the F_1 and F_2 generations for seven characters, but not for clusters/plant or pods/plant. In both F_1s and F_2s separately, the seed yield/plant showed significant positive genotypic and phenotypic correlations with all the characters except days to 50% flowering and seeds/pod. It could be concluded from the present study that the seed yield in green gram could be easily increased by practising selection for plant height, number of primary branches, pods/cluster, pod length and harvest index in both the F_1 and F_2 generations.

Table 4
Correlation coefficients between seed yield (g) and other characters in the F_1 and F_2 generations in 16 green gram crosses

Character	Filial generation	Genotypic correlation coefficient	Phenotypic correlation coefficient	Coheritability
Plant height (cm)	F_1	0.48**	0.31*	0.26
	F_2	0.23*	0.02	0.09
No. of primary branches	F_1	0.65**	0.64**	0.20
	F_2	0.74**	0.19	0.19
Days to 50% flowering	F1	–0.40**	–0.24*	–0.23
	F_2	0.53**	0.81**	0.63
Clusters/plant	F_1	0.89**	0.81**	0.35
	F_2	0.55**	0.43**	0.21
Pods/cluster	F_1	0.45**	0.27*	0.10
	F_2	0.79**	0.36**	0.26
Pods/plant	F_1	0.99**	0.89*	0.23
	F_2	0.52**	0.63**	0.15
Pod length (cm)	F_1	0.31*	0.28*	0.11
	F_2	0.37**	0.23*	0.16
Seeds/pod	F_1	–0.28*	0.06	–0.09
	F_2	–0.35	0.01	–0.10
Harvest index	F_1	0.28*	0.23*	0.19
	F_2	0.31*	0.26*	0.21

*, ** Significant at the $p = 0.05$ and $p = 0.01$ level, respectively

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References

Ahmed, M. S. H., Abd–El–Haleem, S. H. M., Bakheit, M. A., Mohamed, S. M. S. (2008): Comparison of three selection methods for yield and components of three faba bean (*Vicia faba* L.) crosses. *World J. Agric. Sci.,* **4**, 635–639.

Burton, G. W. (1952): Quantitative inheritance of grass. *Proc. 6th Int. Grassland Cong.,* Pennsylvania State College, USA, Vol. 1, pp. 74–83.

Del Rio, A. H., Bamberg, J. B., Huaman, Z. (1997): Assessing changes in the potato gene banks. *Theor. Appl. Genet.,* **95**, 199–204.

Empig, L. T., Lantican, R. M., Escuro, P. B. (1970): Heritability estimates of quantitative characters in mungbean (*Phaseolus aureus* Roxb.). *Crop Sci.,* **10**, 240–241.

Gupta, S., Kumar, S., Singh, B. B. (2004): Relative genetic contributions of ancestral lines to Indian mungbean (*Vigna radiata* L. Wilczek) cultivars based on coefficient of parentage analysis. *Indian J. Genet.,* **64**, 299–302.

Johnson, H. W., Robinson, H. F., Comstock, R. E. (1955): Estimates of genetic and environmental variability in soybean. *Agron. J.,* **47**, 314–318.

Kalita, T., Hazarika, G. N. (1997): Inheritance of yield attributing traits in mungbean. *Indian J. Pulses Res.,* **10**, 49–52.

Medhi, B. N., Hazarika, M. H., Chowdhary, R. K. (1980): Genetic variability and heritability for seed yield components in green gram. *Trop. Grain Leg. Bull.,* **17–18**, 32–34.

Naidu, N. V., Satyanarayana, A. (1991): Studies on genetic divergence over environment in mungbean. *Indian J. Genet.,* **51**, 454–460.

Naik, B. S., Kole, C. (2002): Inheritance of seed protein expression in mungbean. *Indian J. Genet.,* **62**, 79–80.

Nei, M. (1960): *Studies on the Application of Biometrical Genetics to Plant Breeding.* Mum. Coll. Agric., Kyoto University, Kyoto, Japan. pp. 82–100.

Panse, V. G., Sukhatme, P. V. (1957): *Statistical Methods for Agricultural Workers.* ICAR, New Delhi. pp. 48–123.

Parida, D., Singh, D. P. (1984): Association, heritability and genetic advance in the F_2 generation of wide and varietal crosses of green gram. *Madras Agric. J.,* **71**, 351–356.

Ramana, M. V. (1985*): The studies on character association and genetic divergence in mungbean.* MSc thesis. GB Pant Univ. of Agric. Tech., Pantnagar, India. pp. 101–102.

Ramana, M. V., Singh, D. P. (1987): Genetic divergence in mungbean. *Crop Improv.,* **14**, 23–27.

Rehman, A. U., Ali, M. A., Atta, B. M., Saleem, M., Abbas, A., Mallahi, A. R. (2009): Genetic studies of yield related traits in mungbean (*Vigna radiata* L. Wilczek). *Australian J. Crop Sci.,* **3**, 352–360.

Singh D. P., Sharma, B. L. (1981): Evaluation of mungbean germplasm. *Madras Agric. J.,* **68**, 289–295.

Singh, K. B., Malhotra, R. S. (1970): Estimates of genetic and environmental variability in mung (*Phaseolus aureus* Roxb.). *Madras Agric. J.,* **57**, 155–159.

Singh, P., Singh, I. B., Singh, U., Singh, H. G. (1975): Interspecific hybridization between mung (*Phaseolus aureus* Roxb.) and urd (*Phaseolus mungo* L.). *Sci. Cult.,* **41**, 233–234.

Singh, R. K., Chaudhary, B. D. (1985): *Biometrical Methods in Quantitative Genetic Analysis,* Kalyani Publ., Ludhiana, India. pp. 39–54.

Singh, S. P., Singh, H. B., Mishra, S. N., Singh, A. B. (1968): Genotypic and phenotypic correlations among some quantitative characters in mungbean. *Madras Agric. J.,* **55**, 233–237.

Tomar, G. S., Singh, L., Sharma, D. (1972): Effects of environment on character correlation and heritability in green gram. *SABRAO Newsl.,* **4**, 49–52.

Yohe, J. M., Poehlman, J. M. (1975): Regressions, correlations and combining ability in mungbean (*Vigna radiata* L. Wilczek). *Trop. Agric.,* **52,** 343–352.

Yonezawa, K., Yamagata, H. (1981): Selection strategy in breeding of self-fertilizing crop. II: On the use of selection among F_2 populations by the presence and absence of promising phenotypes. *Japanese J. Breeding,* **31**, 215–225.

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INDIRECT SHOOT REGENERATION OF IRANIAN PURPLE CONEFLOWER (*Echinacea purpurea* L.) FROM COTYLEDON AND HYPOCOTYL EXPLANTS

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In vitro plant regeneration was optimized for Iranian purple coneflower via organogenesis from callus cultures derived from cotyledon and hypocotyl tissues by placing them on MS medium supplemented with different concentrations and combinations of BAP and NAA. The experiment was laid out as a completely randomized design in a factorial arrangement with three replications. The results indicated that the mean callus induction was influenced by explant type, with a significant difference between cotyledon (77.81%) and hypocotyl (65.33%) explants at the 0.01 probability level. In relation with the regeneration rate, no significant differences were observed between the two types of explants. For both cotyledons and hypocotyls the optimum shoot regeneration frequency (31.5% and 32.5%, respectively) and number of shoots per explant (5.2 and 5.3, respectively) were achieved using medium supplemented with 0.4 mg l^{-1} BAP. Proliferated shoots were elongated in hormone-free MS medium and well-developed shoots were rooted on MS medium, both with and without the addition of 2 mg l^{-1} IBA. All the plantlets survived acclimatization, producing normal plants under controlled conditions. This study revealed that cotyledon and hypocotyl explants of *E. purpurea* have relatively good potential for callus induction and shoot formation. Furthermore, a beneficial method has been established for the micropropagation of this valuable medicinal species.

Key words: Iranian purple coneflower, plant regeneration, organogenesis, tissue culture

Abbreviations: BAP: 6-benzylaminopurine, IBA: indole-3-butyric acid, NAA: naphthaleneacetic acid

Introduction

Purple coneflower (*Echinacea purpurea* L.), a genus native to North America, belongs to the family *Astereaceae* .It is an important commercial species and has been used as a medicinal plant for hundreds of years (Percival, 2000). Several authors confirmed that the chemical composition of *Echinacea* includes alkamides and caffeic acid derivatives as active compounds, which can be used for the stimulation of the immune system against tumour cells and to act

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as anti-inflammatory agents for the treatment of upper respiratory tract infections (Bauer and Wagner 1991; Bauer 1998; Barrett 2003). Due to market demand and its medicinal properties, interest is increasing in this genus as a new specialty crop. Three species of *Echinacea* are generally used medicinally: *E. purpurea* Moench (roots and tops), *E. angustifolia* DC (roots) and *E. pallida* Nutt (roots) (Perry et al. 2001; Pellati et al. 2004). *E. purpurea* is probably the most widely used and is certainly the most widely cultivated medicinal species of the genus (McKeown 1999).

There are several reports on *in vitro* regeneration in *E. purpurea* via both organogenesis and somatic embryogenesis (Choffe et al., 2000; Harbage, 2001; Koroch et al., 2002; 2003; Lakshmanan et al., 2002; Mechanda et al., 2003; Zobayed and Saxena, 2003; Jones et al., 2007). In early experiments, anther and mesophyll protoplasts were used as explants for the induction of callus that subsequently differentiated into shoots and roots (Pan et al., 2004; Zhu et al., 2005; Zhao et al., 2006). *In vitro* techniques facilitate genetic manipulation and plant multiplication in axenic conditions, but these techniques depend greatly on a reliable regeneration system.

The principal aim of the current study was therefore to determine the role of combinations of NAA and BAP in the induction of regeneration from hypocotyl and cotyledon tissue and to develop an optimized protocol for an efficient micropropagation system for this species.

Materials and methods

Plant material

Seeds of Iranian coneflower, collected in a central region of Iran, were stratified by the dehulling method and then sterilized by immersing in 70% ethanol for 2 min and soaking in 0.1% mercury chloride ($HgCl_2$) containing 0.1% Tween 20 for 7 min and subsequently rinsed three times with sterile distilled water under a laminar flow hood. The sterile seeds were germinated in a basal medium containing MS salts (Murashige and Skoog 1962), B_5 vitamins (Gamborg et al. 1968), 30 g/l sucrose and 7 g/l agar.

Callus induction, shoot regeneration and culture conditions

After 15 days, hypocotyl explants (approx. 1 cm long) and cotyledon sections (5×10 mm) were placed on callus induction medium supplemented with different concentrations of BAP (0, 0.2, 1.2 mg/l) alone or in combination with NAA (0, 0.1, 0.6 mg/l). Shoot induction from calli was carried out on MS medium containing NAA (0, 0.05, 0.3 mg/l) and BAP (0, 0.4, 2.4 mg/l). The pH of all the media was adjusted to 5.8 with NaOH or HCl before adding agar. Medium without plant growth regulators was used as a control. The cultures were incubated in a growth cabinet with a 16-h photoperiod under cool white light (40–60 $\mu mol/m^2/s$) at 25°C and subcultured at 2-week intervals.

Rooting of shoots and acclimatization of plants

The rate of callus formation was determined after 3 weeks. Regeneration was quantified after 5 weeks for all the cultures. After 5 weeks, the regenerants were excised from the explants and subcultured on basal medium in jam jars for the development of plantlets. To promote the development of roots, the plantlets were transferred to MS medium alone or supplemented with IBA (2 mg/l). For acclimatization, rooted shoots were removed from the culture, rinsed in water to remove the medium and transferred to pots filled with a mixture of pasteurized field soil, sand and perlite (1:1:1 by volume) under controlled conditions in a growth chamber set at 25°C and 95% relative humidity with a 16-h photoperiod (30–45 $\mu mol/m^2/s$) for 3 weeks.

Experimental design and statistical analysis

The experiment was laid out as a completely randomized design in a factorial arrangement with three replications, each involving 3 Petri dishes containing 7 explants. Significance was determined by analysis of variance (ANOVA) and the differences between the means were compared by Duncan's multiple range test using the MSTAT-C computer programme (Michigan State University). Data given in percentages were subjected to arcsine ($\sqrt{X}$) transformation before statistical analysis.

Results and discussion

Cotyledon and hypocotyl explants cultured on basal MS medium with different combinations of NAA/BAP exhibited callus formation after 3 weeks of incubation (Table 1). Callus induction and shoot organogenesis were variable and depended on the combination of growth regulators applied. According to analysis of variance (ANOVA) for callus formation and shoot regeneration, there were significant differences between the BAP and NAA concentrations. On the other hand, all the interactions between them were significant at the 0.01 probability level except the explant × NAA interaction for both callus induction and shoot regeneration and the explant × NAA × BAP interaction for shoot regeneration only (Table 2). Callus formation was initiated mainly from the margins of cotyledon explants and at the cut-surface of the hypocotyls (Fig. 1A). In general, the rate of callus induction on cotyledonary leaves (77.81%) was significantly higher than on hypocotyl explants (65.33%).

A similar response was observed between NAA and BAP for callus formation in all the media. Most of the explants formed callus in all concentrations of NAA and BAP. The highest percentage of callus induction occurred with 0.2 mg/l BAP and 0 mg/l NAA for cotyledon segments (97%) and with 0.2 mg/l BAP and 0.6 mg/l NAA for hypocotyls (91%) (Table 1). All the concentrations of NAA were effective for callus induction. Callus developing at higher concentrations (>1.2 mg/l) of BAP was brown and showed necrosis symptoms (Table 1).

Shoot regeneration from calli was observed within 5 weeks of culture (Fig. 1B). The percentage of explants producing shoots and the number of shoots per explant were influenced by the concentrations of BAP and NAA tested ($P<0.01$) (Table 2). Of the various combinations tested, MS medium supplemented with 0.4 mg/l BAP and 0 mg/l NAA was the most effective for shoot regeneration, leading to high shoot regeneration frequency (31.5%) associated with a high number of shoots per explant (5.2) for cotyledon and the highest frequency (32.5%) associated with the greatest number of shoots per explant (5.3) for hypocotyl explants (Table 3). In the presence of more than 1.2 mg/l BAP, the hypocotyl explants exhibited vitrification. This problem was previously reported at high concentrations of this plant growth regulator. Considering both the percentage of explants producing shoots and the number of shoots per explant, the best shoot multiplication was achieved on a range of media supplemented with 0.4 mg/l BAP and 0 mg/l NAA. Furthermore, there was no significant difference between cotyledon and hypocotyl explants for shoot regeneration (Table 2). However, a low concentration of BAP (0.4 mg/l) added to the medium resulted in great stimulation of shoot regeneration.

Table 1
Effect of different combinations of NAA and BAP on callus induction and necrosis from cotyledon and hypocotyl explants of *E. purpurea* after 3 weeks of culture

Growth regulator concentration (mg/l)		% Explants producing callus		% Necrotic callus[2]
NAA	BAP	Cotyledon	Hypocotyl	
0	0	27.33^{f}	52.00^{def}	0^{c}
	0.2	97.00^{a}	63.67^{bcde}	3^{c}
	1.2	88.67^{abc}	38.67^{ef}	21^{a}
0.1	0	60.67^{cde}	63.33^{bcde}	17^{a}
	0.2	94.33^{a}	55.30^{de}	2^{c}
	1.2	77.67^{abcd}	74.67^{abcd}	20^{a}
0.6	0	85.67^{abc}	80.00^{abcd}	8^{b}
	0.2	91.33^{ab}	91.00^{ab}	2^{c}
	1.2	77.67^{abcd}	69.33^{abcd}	11^{b}

Values within a column followed by different letters are significantly different at the 0.01 probability level using Duncan's multiple range test

Table 2
Analysis of variance for callus formation and shoot regeneration traits

S.O.V	df	MS (Mean square)	
		Callus formation	Shoot regeneration
Explant	1	0.210^{**}	0.031^{NS}
NAA	2	0.224^{**}	3.133^{**}
BAP	2	0.191^{**}	14.757^{**}
Explant×NAA	2	0.033^{NS}	0.003^{NS}
Explant×BAP	2	0.133^{**}	1.460^{*}
NAA×BAP	4	0.062^{*}	5.271^{**}
Explant×NAA×BAP	4	0.084^{**}	0.251^{NS}
Error	36	0.021	0.411
CV (%)		20.25	20.12

NS: Non-significant, * and **: Significant at the 0.05 and 0.01 probability levels, respectively

Table 3
Effect of various combinations of NAA and BAP on adventitious shoot regeneration from explants of *E. purpurea* after 5 weeks of culture

Growth regulator concentration (mg/l)		% Explants producing shoots		Mean no.[1] of shoots per explant	
NAA	BAP	Cotyledon	Hypocotyl	Cotyledon	Hypocotyl
0	0	4.2	2.6	0.8^{e}	0.9^{e}
	0.4	31.5	32.5	5.2^{a}	5.3^{a}
	2.4	21.7	21.3	3.5^{bc}	3.6^{bc}
0.03	0	7.3	6.7	2.7^{de}	2.3^{de}
	0.4	17.2	15.8	2.7^{cd}	2.8^{cd}
	2.4	25.9	24.1	3.8^{b}	4.2^{b}
0.3	0	7.6	5.4	2.4^{de}	2.2^{de}
	0.4	20.9	19.1	3.4^{c}	3.3^{c}
	2.4	17.9	16.1	2.3^{cd}	2.8^{cd}

Values within a column followed by different letters are significantly different at the 0.01 probability level

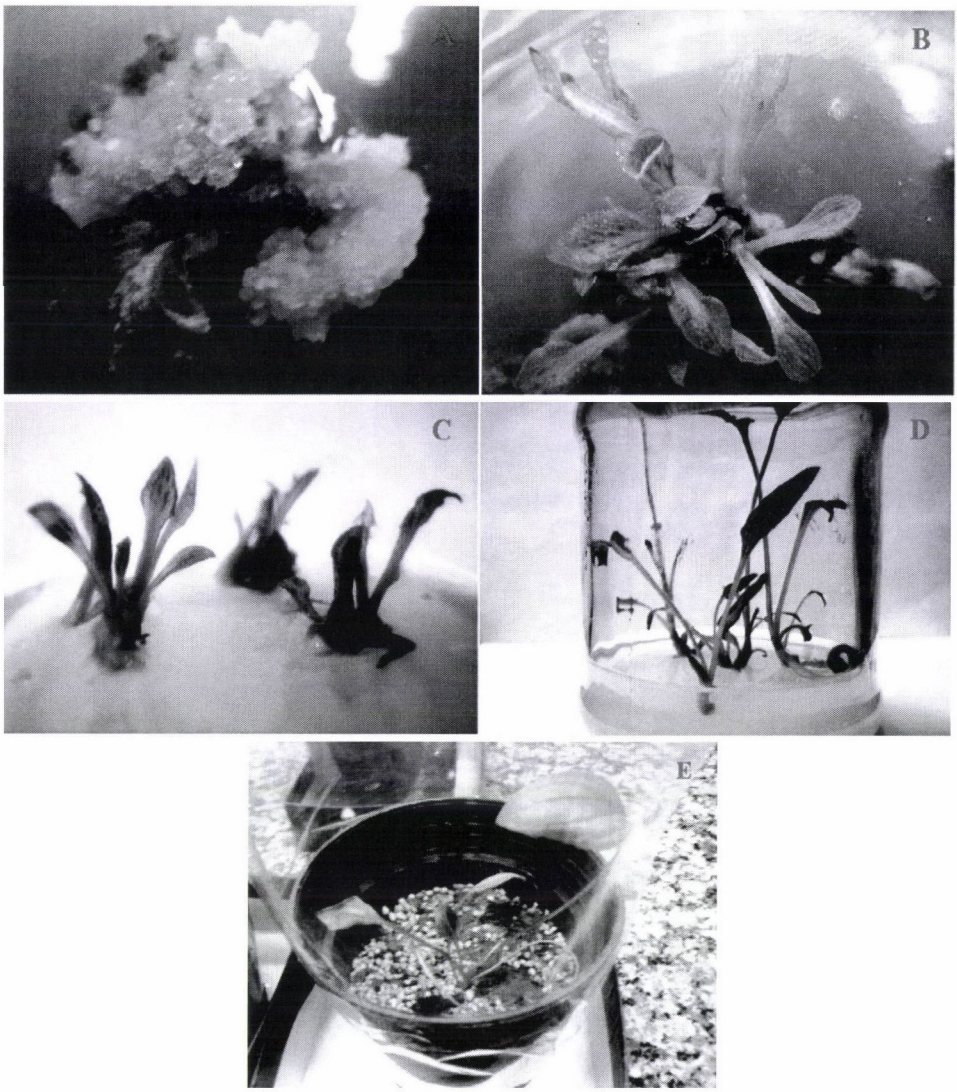

Fig. 1A–E: Adventitious shoot regeneration and *in vitro* shoot development on cotyledon and hypocotyl explants of purple coneflower. A: Callus formation after 3 weeks of culture on a medium supplemented with 0.4 mg/l BAP and 0 mg/l NAA. Callus formation is clearly visible on the margins of the explant and shoot regenerants appear at the callus interface. B: Adventitious (multiple) shoots developed from cotyledon segments after 5 weeks of culture. C: Regenerated shoots transferred to hormone-free MS medium for developing and rooting (after 5 weeks). D: Regenerated shoots transferred to hormone-free MS medium for developing and rooting (after 6 weeks). E: Establishment of plants under controlled conditions

The rooting of regenerated, elongated shoots was observed after 2–3 weeks of culture. The percentage of rooted shoots fluctuated between 15 and 20%. Roots developed from the basal end of shoots, and rooting only occurred in medium with 2 mg/l IBA (data not shown). All the rooted plantlets survived after 3 weeks under controlled conditions (Fig. 1C, D). The plantlets were cultured in pots containing perlite, then transferred to soil and grown to maturity (Fig. 1E).

In the present study, a mixed method was used for seed sterilization. First the seeds were dehulled to remove the seed coat layers in order to overcome seed dormancy and remove surface contamination. They were then sterilized as described in the Materials and methods section. Various methods have proved successful for seed sterilization, including surface sterilization with ethanol and sodium hypochloride (Lakshmanan et al., 2002; Mechanda et al., 2003; Zobayed and Saxena, 2003) along with the detergent Tween 20 (Choffe et al., 2000; Koroch et al., 2002). Alternately, removing the seed coat layers to prevent the contamination of the seeds was proposed by Harbage (2001). Similar results were obtained in the present study.

Combinations of BAP and NAA were reported to induce regeneration of whole plantlets via indirect shoot organogenesis from leaf explants of *E. pallida* and *E. purpurea*. Koroch et al. (2002; 2003) found that leaves of *E. purpurea* and *E. pallida* had great organogenic potential for shoot formation, which is directly related to the combination of exogenous growth regulators in the culture medium. They also suggested that the balance of auxins and cytokinins is a decisive morphogenic factor. The present results partially support these reports and clearly underline the importance of combining NAA and BAP for efficient organogenesis from cultured cotyledonary leaves and hypocotyl explants.

In other studies, plant regeneration was obtained from petioles of *E. purpurea* via indirect organogenesis using a small amount of BAP (Choffe et al., 2000; Wang and To, 2004). The results presented here are in agreement with these findings, as it was observed that a low concentration of BAP and NAA was efficient for the induction of callus and subsequently shoot regeneration. In the present study, hypocotyl and cotyledon tissues showed a similar response to BAP as regards shoot regeneration.

Various combinations of BAP with NAA were effective in inducing callus and shoot organogenesis from hypocotyl and cotyledon explants, as previously reported by Bhatti et al. (2002) for three species of the *Echinaceae* genus: *E. angustifolia, E. purpurea* and *E. pallida.* The multiple shoot induction rate and morphogenetic response varied significantly according to the explant type and plant growth regulator concentration (Uranbey, 2005).

In addition, *E. purpurea* was found to be largely recalcitrant to regeneration (Choffe et al., 2000). A noteworthy observation of this study was the indirect development of shoots. Indirect organogenesis is defined as the formation of calli on explants and subsequently the development of shoots

(Sharp et al., 1986). The selection of a suitable explant at the correct development stage plays a key role in the successful establishment of culture under *in vitro* conditions. The morphological integrity of the explant along with the proper choice of plant growth regulators strongly influence the induction of optimal callus and shoot regeneration (Khawar et al., 2005).

The most significant results of the present study were the development of *in vitro* protocols for the induction of callus and shoot organogenesis from hypocotyl and cotyledon explants of *E. purpurea* exposed to different auxin and cytokinin concentrations. The cytokinin BAP in low concentrations was found to be the most effective for the induction of shoot regeneration. Furthermore, this research demonstrated that cotyledon tissue of *E. purpurea* is competent for callus formation and that both hypocotyl and cotyledon explants are capable of regeneration. The micropropagation system for *E. purpurea* was optimized. In view of the demand for highly valuable chemicals such as antitumour substances, the large-scale propagation of *E. purpurea* using a plant regeneration technique capable of producing multiple clonal plants *in vitro* is greatly to be desired.

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References

Barrett, B. (2003): Medicinal properties of *Echinacea*: a critical review. *Phytomedicine*, **10**, 66–86.

Bauer, R. (1998): The *Echinacea* story, the scientific development of an herbal immunostimulant. pp. 317–332. In: Prendergast, H. D. V., Etkin, N. L., Harris, D. R., Haughton, P. J. (eds.), *Plants for Food and Medicine. Modern Treatment and Traditional Remedies*. The Royal Botanic Gardens, Kew,

Bauer, R., Wagner, H. (1991): *Echinacea* species as potential immunostimulatory drugs. pp. 253–321. In: Wagner, H., Farnsworth, N. R. (eds.), *Economic and Medicinal Plant Research*. Academic, New York,

Bhatti, S. M., Myles, E. L., Long, D. E., Sauve, R. (2002): *In vitro* regeneration of St. Johns wort and coneflowers. *SNA Research Conference*, **47**, 340–342.

Choffe, K. L., Victor, J. M. R., Murch, S. J., Saxena, P. K. (2000): *In vitro* regeneration of *Echinacea purpurea* L.: Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. *In vitro Cell Dev. Biol. Plant.*, **36**, 30–36.

Gamborg, O. L., Miller, R. A., Ojima, K. (1968): Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.*, **50**, 151–158.

Harbage, J. F. (2001): Micropropagation of *Echinacea angustifolia, E. pallida,* and *E. purpurea* from stem and seed explants. *Hort. Sci.*, **36**, 360–364.

Jones, M. P. A., Yi, Z., Murch, S. J., Saxena, P. K. (2007): Thidiazuron-induced regeneration of *Echinacea purpurea* L.: Micropropagation in solid and liquid culture systems. *Plant Cell Rep.*, **26**, 13–19.

Khawar, K. M., Sarihan, E., Sevimay, C., Çöçü, S., Parmaksiz, I., Uranbey, S., Ipek, A., Kaya, M. D., Sancak, C., Özcan, S. (2005): Adventitious shoot regeneration and micropropagation of *Plantago lanceolata* L. *Period Biol.,* **107**, 113–116.

Koroch, A. R., Juliani, H. R., Kapteyn, J., Simon, J. E. (2002): *In vitro* regeneration of *Echinacea purpurea* from leaf explants. *Plant Cell Tiss. Org. Cult.,* **69**, 79–83.

Koroch, A. R., Kapteyn, J., Juliani, H. R., Simon, J. E. (2003): *In vitro* regeneration of *Echinacea pallida* from leaf explants. *In vitro Cell Dev. Biol. Plant.,* **39**, 415–418.

Lakshmanan, P., Danesh, M., Taji, A. (2002): Production of four commercially cultivated *Echinacea* species by different methods of *in vitro* regeneration. *J. Hortic. Sci. Biotechnol.,* **77**, 158–163.

McKeown, K. A. (1999): A review of taxonomy of the genus *Echinacea.* pp. 482–490. In: Janick, J. (eds.), *Perspectives on New Crops and New Uses*. Purdue University, West Lafayette,

Mechanda, S. M., Baum, B. R., Johnson, D. A., Arnason, J. T. (2003): Direct shoot regeneration from leaf segments of mature plants of *Echinacea purpurea* (L.) Moench. *In vitro Cell Dev. Biol. Plant.,* **39**, 505–509.

Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.,* **15**, 473–497.

Pan, Z. G., Liu, C. Z., Zobayed, S. M. A., Saxena, P. K. (2004): Plant regeneration from mesophyll protoplasts of *Echinacea purpurea. Plant Cell Tiss. Org. Cult.,* **77**, 251–255.

Pellati, F., Benvenuti, S., Magro, L., Melegari, M., Soragni, F. (2004): Analysis of phenolic compounds and radical scavenging activity of *Echinacea* species. *J. Pharm. Biomed. Anal.,* **35**, 289–301.

Percival, S. S. (2000): Use of *Echinacea* in medicine. *Biochem Pharmacol.,* **60**, 155–158.

Perry, B., Burges, E., Glennie, V. (2001): *Echinacea* standardization: analytical methods for phenolic compounds and typical levels in medicinal species. *J. Agric. Food Chem.,* **49**, 1702–1706.

Sharp, W. R., Sondhal, M. R., Caldar, R. S., Maraffa, S. B. (1986): The physiology of *in vitro* asexual embryogenesis. *Hortic. Rev.,* **2,** 268–310.

Uranbey, S. (2005): Thidiazuron induced adventitious shoot regeneration in henbane (*Hyoscyamus niger* L.). *Biol. Plant.,* **49**, 427–430.

Wang, H. M., To, K. Y. (2004): *Agrobacterium*-mediated transformation in the high value medicinal plant *Echinacea purpurea. Plant Sci.,* **166**, 1087–1096.

Zhao, F. C., Nilanthi, D., Yang, Y. S., Wu, H. (2006): Anther culture and haploid plant regeneration in purple coneflower (*Echinacea purpurea* L.). *Plant Cell Tiss. Org. Cult.,* **86**, 55–62.

Zhu, L. Q., Wang, B. C., Zhou, J., Chen, L. X., Dai, C. Y., Duan, C. R. (2005): Protoplast isolation of callus in *Echinacea augustifolia. Colloids Surfaces B. Biointerfaces*, **44**, 1–5.

Zobayed, S. M. A., Saxena, P. K. (2003): *In vitro* regeneration of *Echinacea purpurea* L.: Enhancement of somatic embryogenesis by indolebutyric acid and dark pre-incubation. *In vitro Cell Dev. Biol. Plant.,* **39**, 605–612.

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EFFECT OF DIGESTED DISTILLERY SPENT WASH ON NODULATION, NUTRIENT UPTAKE AND PHOTOSYNTHETIC ACTIVITY IN CHICKPEA (*Cicer arietinum*)

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This work investigated the effect of graded doses and methods of application of digested spent wash on seed germination, nodulation, photosynthetic activity and nutrient uptake in chickpea and on soil properties. Under laboratory conditions, lower concentrations of digested spent wash were not inhibitory to seed germination, whereas higher concentrations led to poor seedling growth and delayed seed germination. However, under greenhouse conditions, seed germination was slightly better at higher concentrations. Increased concentrations of digested spent wash affected the nodulation of chickpea. Irrigation with digested spent wash in pots had an adverse effect on nodulation as compared to its soil application. Lower concentrations of digested spent wash had no detrimental effect on plant growth (shoot length, root length and their weight). The photosynthetic activity of chickpea plants, measured as chlorophyll *a* fluorescence, was maximum at 10% and 100 m^3 ha^{-1} of digested spent wash, while a decrease was observed at higher concentrations. With an increase in the concentration of digested spent wash, there was a decrease in N and P uptake by chickpea plants. No significant difference was observed in soil pH, but the EC, organic carbon and total N and P contents of post-harvest soil increased with an increase in the concentration of digested spent wash.

Key words: digested spent wash, seed germination, nodulation, nutrient uptake, plant growth, chlorophyll *a* fluorescence, chickpea

Introduction

Distillery units producing alcohol are major revenue-earning enterprises. Alcohol is produced from sugarcane molasses. The molasses is fermented with yeast and alcohol is distilled from fermented wash, leaving behind a large volume of foul-smelling coloured waste water, generally known as spent wash or distillery effluent. The proportion of spent wash is nearly 14–15 times the total alcohol production. As there are large numbers of distilleries spread all over

the world, large quantities of this effluent are produced (Manonmani et al., 1990, Raghukumar et al., 2004). The disposal of large quantities of distillery effluent poses environmental problems, as it contains a considerable amount of organic material and has high biological oxygen demand (BOD) and chemical oxygen demand (COD). Due to the high BOD of raw spent wash, the application of an anaerobic treatment technology with biogas recovery has been reported to be highly effective (Nandy et al., 2002). A large number of microbes have been screened for the decolorization of digested spent wash (Gad and El Sayaad, 2010; Seyis and Subasioglu, 2009). Anaerobic treatment is an accepted practice and various high - rate anaerobic reactor designs have been tried at the pilot and full scale operation level. However, anaerobically treated spent wash still contains high concentrations of organic pollutants and cannot be discharged directly. Being of plant origin, spent wash contains large quantities of soluble organic matter and plant nutrients, which if utilized for crop production could prove to be a good source of nutrients. This is expected to solve the problem of waste disposal.

The only problem with distillery spent wash is that it contains a significant quantity of salts, so long-term use may affect the physico-chemical properties of the soil. To minimize the detrimental effect of spent wash on crop ecology, it is necessary to understand its exact chemical composition. It has been revealed that factors such as pH, electrical conductivity (EC), BOD, COD and the organic C, N, P and K contents of spent wash may affect plant growth (Mahimairaja and Bolan, 2004). Studies have shown that non-judicious use of spent wash may adversely affect crop growth and soil properties by increasing soil salinity. The farmers around distillery units use distillery spent wash as a source of nutrients for crop production without knowing the proper method of application. Agricultural scientists are therefore deeply concerned about its use or disposal on agricultural land, which is a limited commodity. The adverse effect of distillery spent wash on soil processes such as organic matter decomposition, nutrient mineralization, nitrogen fixation and sulphur oxidation may lead to reduced levels of plant nutrients in the soil and in the crop, which could ultimately affect the photosynthetic activity.

It is therefore necessary to find ways of using distillery spent wash without causing pollution hazards, making it a potential substance for crop irrigation and nutrition. To establish the dilution at which digested spent wash can be used in irrigation and as a nutrient source without adversely affecting soil and crop health, the present investigation was undertaken to study the effect of graded doses and the method of application of digested spent wash on the seed germination, nodulation, nutrient uptake and photosynthetic activity of chickpea and on soil properties.

Materials and methods

Digested spent wash

The digested spent wash was collected from an anaerobic treatment plant (Panipat Distillery India Ltd.) in plastic cans and stored at room temperature. This spent wash was used for all the experiments throughout the study after proper dilution. The pH, EC, total solids, ash content, volatile solids and percentage carbon were determined by standard methods. The chemical oxygen demand of the digested spent wash was determined by the standard potassium dichromate method (Anonymous, 1986).

Effect of digested spent wash on seed germination of chickpea under laboratory conditions

Seeds of chickpea (var. HC-5) were surface sterilized with mercuric chloride (Vincent, 1970) and were spread on sterilized petri dishes lined with a double layer of filter paper saturated with equal volumes (5 ml) of different concentrations (0, 2.5, 5.0, 10, 20, 50 and 100%) of digested spent wash. For each treatment, there were three replicates with 10 seeds in each replicate. Plates were incubated in an incubator at 28 ± 2°C and seed germination was observed at different time intervals.

Effect of digested spent wash on nodulation, nutrient uptake and plant growth of chickpea

The recommended dose of fertilizers (RDF) (100% RDF = 20 kg N + 40 kg P_2O_5 hectare^{-1}) was varied to reveal the beneficial effect of supplementation with digested spent wash at levels ranging from 2.5% to 50% along with irrigation. Similarly, the soil was amended with different levels (10, 25, 50, 100, 250 and 500 m^3 ha^{-1}) of digested spent wash before sowing.

A green-house experiment was conducted using chickpea (var. HC-5) as a test crop. The seeds were inoculated with *Mesorhizobium* sp. strain CH 1233 and sown in pots each containing a measured quantity of soil (3 kg). Soil analysis for pH, EC and residual C, N and P was done before and after the harvest of the chickpea crop. The initial soil analysis showed that the soil was of sandy loam type. The pH of the soil was 8.01 and electrical conductivity (EC) was 0.11 dS m^{-1}. The organic carbon and total N contents were 0.202% and 0.023%, respectively. The total nitrogen in the soil was estimated by the Kjeldahl method (Bremner, 1960), the total phosphorus content by the method of John (1970) and the organic carbon content by the wet digestion method (Walkley and Black, 1934).

The pots were irrigated or amended with water or digested spent wash at the rates given above. Digested spent wash was applied either as a soil amendment before the sowing of the crop or was applied along with irrigation every 3 or 4 days throughout the growth of the chickpea plants to avoid the accumulation of higher doses of the digested spent wash. After 60 days of plant growth, sample plants were uprooted and observations were made on nodule number and dry weight. The root and shoot biomass and the total N and P contents in the shoots were estimated after 90 days of plant growth. The nodules and plants were dried in an oven at 80°C for 3 days till constant weight. The N and P contents of the shoots were estimated as described above for the soil, except that the quantity of plant sample used was 200 mg instead of 500 mg. Chlorophyll *a* fluorescence was measured using a compact, portable Plant Efficiency Analyzer (PEA). Chlorophyll *a* fluorescence was determined in all the replicates with 20 measurements (Dudeja and Chaudhary, 2005). The fully expanded leaves were first acclimated to the dark for at least 2 min by fixing clips. The dark-adapted samples were continuously irradiated for 1 s (1500 μmol m^{-2} s^{-1}) provided by an array of three light-emitting diodes in the sensor. The fluorescence signals were detected using a *PIN* photodiode after passing through a long pass filter (50% transmission at 720 nm). The first reliable point of the fluorescence transient was measured at $t_0 = 0.05$ μs after the onset of irradiation and was taken as F_0. The data were analysed using the Biolyser 4.0 software programme developed and supplied free of cost by R. Maldonado Rodriguez (Bioenergetics Laboratory at the University of Geneva, Switzerland) using the JIP test (Strasser et al., 2000), which provides parameters indicating the efficiency of photosystem II.

Results

In order to allow spent wash to be applied with minimal detrimental effect on crop growth, the chemical composition of digested spent wash was determined. The digested (treated) spent wash was reddish brown in colour, had an unpleasant smell of burnt sugar and the physico-chemical properties given in Table 1. The effect of this digested spent wash on the seed germination of chickpea showed that lower concentrations (2.5 to 20%) of digested spent wash did not inhibit seed germination under laboratory conditions. Though 100% seed germination was observed at 50% spent wash concentration, it led to poor seedling growth and delayed seed germination (Table 2 and Fig. 1). The effect of digested spent wash on the seed germination of chickpea was also observed in pots, as soil is known to mitigate the adverse effect of digested spent wash applied as soil amendment or with irrigation. All the seeds germinated when the pots were irrigated with digested spent wash up to 20%, but the seeds failed to germinate at a higher concentration (50%) of digested spent wash (Table 3). Similarly, when soil was amended with different concentrations of digested spent wash at the pre-sowing stage, seed germination was observed up to a digested spent wash concentration of 250 m^3 ha^{-1} (Table 3). A higher concentration (500 m^3 ha^{-1}) of digested spent wash completely suppressed seed germination. Since there was no seed germination, no plants were available at higher concentrations (50% and 500 m^3 ha^{-1}) of digested spent wash, so data on nodulation, plant growth, nutrient uptake and photosynthetic activity were not recorded at these concentrations.

The effect of digested spent wash on the nodulation of chickpea was observed as a decrease in nodule number and weight with an increase in the concentration of digested spent wash. However, the lowest concentration (2.5%) of digested spent wash did not inhibit nodulation. In the case of soil amendment, there was no adverse effect on nodulation up to 100 m^3 ha^{-1} of digested spent wash, and in fact an increase in nodule number was observed up to 25 m^3 ha^{-1} of digested spent wash (36.7 nodules $plant^{-1}$) when the soil was amended (10 to 250 m^3 ha^{-1} of digested spent wash) at the pre-sowing stage. There was a decrease in nodule number (14.7 nodules $plant^{-1}$) and weight (17 mg) in soil amended with a higher concentration (250 m^3 ha^{-1}) of digested spent wash (Table 4).

The growth of chickpea plants was recorded as root and shoot weight $plant^{-1}$. Both root weight (0.895 mg) and shoot weight (0.668 mg) were maximum at the 2.5% concentration of digested spent wash. As the concentration of digested spent wash increased, the root and shoot weight decreased (Table 4). The pre-sowing application of digested spent wash up to 50 m^3 ha^{-1} had a beneficial effect on root and shoot weight, but at higher concentrations (100 and 250 m^3 ha^{-1}), a decrease in both root and shoot weight was observed (Table 4). Further, it was seen that root growth was more severely affected than shoot growth by the repeated application of digested spent wash (irrigation) in comparison to a single application (pre-sowing amendment).

Table 1
Physico-chemical characteristics of digested spent wash collected from Panipat distillery

Parameters	Content
Colour	Reddish brown
Smell	Unpleasant
pH	7.92
EC	20.5 dS m^{-1}
COD	20,800 mg L^{-1}
Total solids	21.2 g L^{-1}
Volatile solids	6.8 g L^{-1}
Ash content	14.4 g L^{-1}
Total carbon	3.94 g L^{-1}
Total nitrogen	1.21 g L^{-1}
Total phosphorus	0.076 g L^{-1}

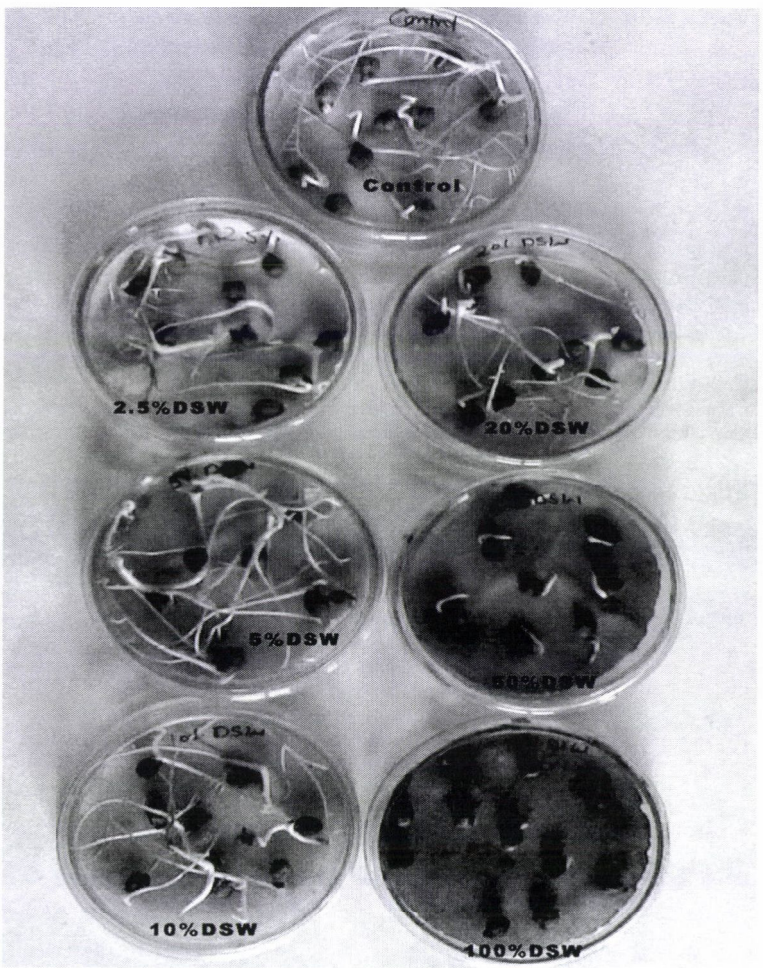

Fig 1. Effect of digested spent wash on seed germination of chickpea under laboratory conditions

Table 2
Effect of digested spent wash on seed germination and seedling growth of chickpea under laboratory conditions

Digested spent wash concentration (%)	% germination	Seedling growth (mm)
0.0	100	82.6
2.5	100	55.4
5.0	100	62.8
10	100	56.0
20	100	53.1
50	100	22.1
100	20	10.2
$CD_{5\%}$		5.7

Table 3
Effect of digested spent wash on seed germination of chickpea under greenhouse conditions

Code	Treatment	% germination
A: Applied along with irrigation		
T1	Control[1]	94
T2	100% RDF[2]	100
T3	75% RDF	100
T4	50% RDF	100
T5	*Mesorhizobium* inoculation[3]	100
T6	*Mesorhizobium* + 2.5% DSW[4]	100
T7	*Mesorhizobium* + 5.0% DSW	100
T8	*Mesorhizobium* + 10% DSW	100
T9	*Mesorhizobium* + 20% DSW	100
B: Applied as soil amendment		
T10	*Mesorhizobium* + 10 m^3 ha^{-1} DSW[5]	88.88
T11	*Mesorhizobium* + 25 m^3 ha^{-1} DSW	100
T12	*Mesorhizobium* + 50 m^3 ha^{-1} DSW	100
T13	*Mesorhizobium* + 100 m^3 ha^{-1} DSW	100
T14	*Mesorhizobium* + 250 m^3 ha^{-1} DSW	83.33

[1]No fertilizer or digested spent wash; irrigated with tap water; [2]Recommended dose of fertilizer (100% RDF = 20 kg N and 40 kg P ha^{-1}); irrigated with water; [3]*Mesorhizobium* inoculation; irrigated with water; [4]*Mesorhizobium* inoculation; irrigated with digested spent wash (DSW); [5]*Mesorhizobium* inoculation + Soil amendment at pre-sowing stage with digested spent; wash; irrigated with water

Applying digested spent wash as irrigation led to a decrease in the N and P uptake of chickpea plants with an increase in the concentration of digested spent wash. The N uptake (26.5 mg $plant^{-1}$) and P uptake (1.64 mg $plant^{-1}$) were maximum when 2.5% of digested spent wash was applied, while these values were 16.6 mg $plant^{-1}$ and 0.88 mg $plant^{-1}$, respectively, at the 20% application rate.

Table 4
Effect of digested spent wash on plant growth of chickpea under greenhouse conditions

Code	Treatment	Nodules plant^{-1}	Nodule dry wt (mg plant^{-1})	Root wt (g plant^{-1})	Shoot dry wt (g plant^{-1})	N uptake (mg plant^{-1})	P uptake (mg plant^{-1})
A: Applied along with irrigation							
T1	Control[1]	6.6	5.00	0.325	0.350	10.0	0.66
T2	100% RDF[2]	5.4	4.30	2.207	1.218	38.0	2.59
T3	75% RDF	6.3	5.80	1.541	0.998	37.2	2.13
T4	50% RDF	4.1	4.20	0.661	0.493	21.0	2.06
T5	*Mesorhizobium* inoculation[3]	24.3	36.10	0.775	0.563	16.4	1.50
T6	*Mesorhizobium* + 2.5% DSW[4]	25.2	35.00	0.895	0.668	26.5	1.64
T7	*Mesorhizobium* + 5.0% DSW	21.5	27.00	0.652	0.540	21.8	1.21
T8	*Mesorhizobium* + 10% DSW	12.8	16.00	0.202	0.442	18.3	0.94
T9	*Mesorhizobium* + 20% DSW	5.9	5.20	0.035	0.441	16.6	0.88
	$CD_{5\%}$	2.6	2.20	0.145	0.168	4.0	0.18
B: Applied as soil amendment							
T10	*Mesorhiz.* + 10 m^3 ha^{-1} DSW[5]	33.6	34.00	0.580	0.580	18.7	0.88
T11	*Mesorhiz.* + 25 m^3 ha^{-1} DSW	36.7	36.00	1.020	0.654	22.4	1.01
T12	*Mesorhiz.* + 50 m^3 ha^{-1} DSW	30.0	31.00	1.131	0.697	27.4	1.21
T13	*Mesorhiz.* + 100 m^3 ha^{-1} DSW	28.2	33.00	0.630	0.548	23.6	0.90
T14	*Mesorhiz.* + 250 m^3 ha^{-1} DSW	14.7	17.00	0.410	0.428	11.8	0.68
	$CD_{5\%}$	3.6	2.73	0.163	0.170	4.9	0.12

Notes 1–5 see Table 3

Soil amendment with digested spent wash at the pre-sowing stage resulted in increased nutrient (N and P) uptake up to a concentration of 50 m^3 ha^{-1}, after which there was a decrease in nutrient uptake. The nitrogen uptake amounted to 18.7, 22.4 and 27.4 mg plant^{-1} in soil amended with 10, 25 and 50 m^3 ha^{-1} of digested spent wash, respectively. The corresponding values for the P uptake of chickpea plants were 0.88, 1.01 and 1.21 mg plant^{-1}. The lowest nutrient uptake of N (11.8 mg plant^{-1}) and P (0.68 mg plant^{-1}) in chickpea was observed at the 250 m^3 ha^{-1} concentration (Table 4).

Chlorophyll *a* fluorescence was found to be maximum at the 10% concentration of digested spent wash, while a slight decrease in chlorophyll *a* fluorescence was observed when the plants were irrigated with 20% digested spent wash (Fig. 2). No major difference was observed in the chlorophyll *a* fluorescence of chickpea plants in the other treatments. When the soil was amended at the pre-sowing stage, maximum chlorophyll *a* fluorescence was observed at 100 m^3 ha^{-1} of digested spent wash (Fig. 3). At the 250 m^3 ha^{-1} concentration, a slight decline in chlorophyll *a* fluorescence was observed, but no significant change was observed in the other treatments.

To determine the effect of applying digested spent wash along with irrigation or as soil amendment on various soil properties, the chemical and physical properties of the soil and its nutrient status were determined before and after the chickpea crop. With increasing amounts of digested spent wash in the irrigation water, there was an increase in the pH of the soil, though the increase

was statistically non-significant. The electrical conductivity (EC) of the soil increased at higher concentrations of digested spent wash, with values of 0.202 dS m^{-1} at 2.5% concentration and 0.553 dS m^{-1} at 50% concentration in comparison to the control (EC 0.111 dS m^{-1}), where the plants were irrigated with water. A single application (pre-sowing amendment) of treated spent wash did not raise the EC of the soil beyond 0.262 dS m^{-1} even at the 500 m^3 ha^{-1} rate.

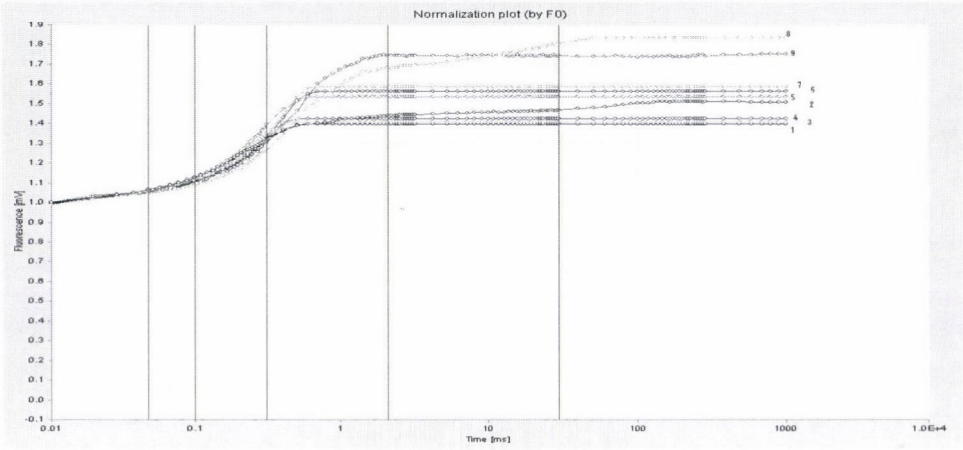

Fig. 2. Effect of digested spent wash (applied along with irrigation) on chlorophyll *a* fluorescence of chickpea plants; 1. Control, 2. 100% RDF, 3. 75% RDF, 4. 50% RDF, 5. *Mesorhizobium* inoculation, 6. *Mesorhizobium* + 2.5% DSW, 7. *Mesorhizobium* + 5.0% DSW, 8. *Mesorhizobium* + 10% DSW, 9. *Mesorhizobium* + 20% DSW

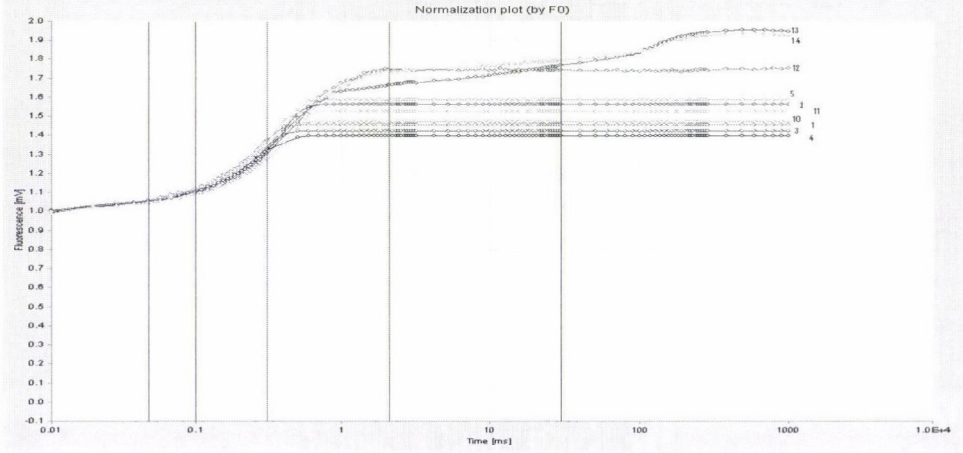

Fig. 3 Effect of digested spent wash (applied as soil amendment) on chlorophyll *a* fluorescence of chickpea plants; 1. Control, 2. 100% RDF, 3. 75% RDF, 4. 50% RDF, 5. *Mesorhizobium* inoculation, 6. *Mesorhizobium* + 10 m^3 ha^{-1} DSW, 11. *Mesorhizobium* + 25 m^3 ha^{-1} DSW, 12. *Mesorhizobium* + 50 m^3 ha^{-1} DSW, 13. *Mesorhizobium* + 100 m^3 ha^{-1} DSW, 14. *Mesorhizobium* + 250 m^3 ha^{-1} DSW

With an increase in the concentration of digested spent wash, either used along with irrigation or as soil amendment, there was a significant increase in the organic C, and total N and P contents in the soil compared with the control after the harvesting of chickpea (Table 4). The soil organic C and N were maximum (0.529 and 0.079%) when the soil was irrigated with 50% digested spent wash, when the phosphorus content was 238.0 mg kg^{-1} soil. When spent wash was applied as a soil amendment, the organic C and N contents were 0.495 and 0.065%, respectively, at the 500 m^3 ha^{-1} level, when the P content was 183.4 mg kg^{-1} of soil (Table 5).

Table 5
Effect of digested spent wash on soil properties

Code	Treatment	pH	EC (dS m^{-1})	OC%	N%	P (mg g^{-1})
A: Applied along with irrigation						
T1	Control soil[1]	8.02	0.111	0.202	0.010	135.3
T2	100% RDF[2]	8.08	0.167	0.277	0.056	172.4
T3	75% RDF	8.06	0.156	0.210	0.050	167.5
T4	50% RDF	8.05	0.132	0.207	0.033	150.7
T5	*Mesorhizobium* inoculation[3]	8.04	0.131	0.212	0.030	148.4
T6	*Mesorhizobium* + 2.5% DSW[4]	8.11	0.202	0.332	0.050	158.4
T7	*Mesorhizobium* + 5.0% DSW	8.24	0.248	0.382	0.053	182.3
T8	*Mesorhizobium* + 10% DSW	8.27	0.344	0.427	0.059	197.4
T9	*Mesorhizobium* + 20% DSW	8.30	0.409	0.447	0.062	218.8
T10	*Mesorhizobium* + 50% DSW	8.32	0.553	0.529	0.079	238.0
	$CD_{5\%}$	NS	0.060	0.027	0.019	11.7
B: Applied as soil amendment						
T11	*Mesorhizobium* + 10 m^3 ha^{-1} DSW[5]	8.19	0.137	0.307	0.048	151.8
T12	*Mesorhizobium* + 25 m^3 ha^{-1} DSW	8.21	0.145	0.322	0.045	167.4
T13	*Mesorhizobium* + 50 m^3 ha^{-1} DSW	8.22	0.147	0.375	0.040	175.9
T14	*Mesorhizobium* + 100 m^3 ha^{-1} DSW	8.25	0.150	0.410	0.056	162.3
T15	*Mesorhizobium* + 250 m^3 ha^{-1} DSW	8.27	0.177	0.442	0.058	178.6
T16	*Mesorhizobium* + 500 m^3 ha^{-1} DSW	8.30	0.262	0.495	0.065	183.4
	$CD_{5\%}$	NS	0.012	0.027	0.018	11.8

Notes 1–5 see Table 3; NS: Non-significant

Discussion

All over the world distilleries generate huge quantities of spent wash/effluent and in spite of major efforts, no comprehensive treatment has yet been elaborated. Even with the best available technology, most of the treated effluent discharged into streams, rivers and natural waters is black, foul-smelling and has very high levels of BOD, COD, TDS and soluble salts. Since distillery spent wash contains a considerable amount of plant nutrients and organic matter, the present investigation was undertaken to explore the possibility of applying digested spent wash to improve chickpea crop productivity.

The application of digested distillery effluent at a concentration of more than 20% had an inhibitory effect on seed germination under laboratory conditions. Similar results were reported in the case of rice, sorghum, cowpea, wheat, soybean and pea (Sahai et al., 1983; Singh et al., 1985; Singh and Bahadur, 1995; Pandey et al., 2007; Deora et al., 2008). Though Ramana et al. (2002a) reported no inhibitory effect of spent wash on the seed germination of various vegetable crops, the seeds failed to germinate at higher concentrations. Therefore, due care should be taken before using spent wash for irrigation purposes.

In the present study, seed germination under pot culture conditions in soil showed that digested spent wash had no adverse effect up to a concentration of 20% (irrigation) or 250 m^3 ha^{-1} (pre-sowing amendment). However, at higher concentrations (50% and 500 m^3 ha^{-1}) it was inhibitory. The application of digested spent wash at higher doses resulted in poor development and establishment of plants. Other authors also reported that higher concentrations of effluent/spent wash retarded the rate of germination and seedling growth in soil (Sahai et al., 1983). A high content of cations, anions and total dissolved solids has been reported to retard seed germination by enriching the salinity and conductivity of the solutes absorbed by the seed prior to germination (Sahai et al., 1983; Singh and Bahadur, 1995; Pandey et al., 2007).

An increased concentration of digested spent wash affected the nodulation of chickpea, to an extent depending on whether the spent wash was applied as a soil amendment or with irrigation water. The addition of digested spent wash to the irrigation water affected nodulation even at a low concentration of 5%, whereas the application of digested spent wash in soil at a rate of up to 100 m^3 ha^{-1} had no adverse effect on nodulation. It is not yet clear precisely which stage of nodule formation is more sensitive to a high concentration of digested spent wash in any legume. Since the root biomass was adversely affected in chickpea, it is quite possible that the toxic components of spent wash might have affected the population of *Rhizobium* and decreased root hair formation. A reduction in nodulation has been reported in various legumes after the application of distillery waste water (Juwarkar et al., 1990; Ramana et al., 2002b; Bhalerao et al., 2004).

Lower doses of digested spent wash (5% or 50 m^3 ha^{-1}) were found to have no adverse effect on the root and shoot growth of chickpea and even proved to be stimulatory. Other researchers also reported the positive effect of diluted spent wash on the crop growth of non-legumes (Zalawadia et al., 1997; Singh et al., 1985; Banerjee et al., 2004; Singh and Bahadur, 1997; Sukanya and Meli, 2004a). The better performance of chickpea at lower concentrations of digested spent wash (irrigation/pre-sowing amendment) can be attributed to its manuring effect. This was further supported by the nutrient content in post-harvest soil and its uptake.

Chlorophyll *a* fluorescence is an indicator of photosystem II efficacy and this was maximum in chickpea at the 10% concentration level (irrigation) and at 100 m^3 ha^{-1} (soil amendment) of digested spent wash. Sahai et al. (1983), on the other hand, reported decreased chlorophyll *a* and chlorophyll *b* contents at higher spent wash concentrations. Similarly, a higher distillery spent wash concentration had an inhibitory effect on the chlorophyll content of *Phaseolus aureus,* while lower concentrations (1–10%) increased chlorophyll (Chandra et al., 2004).

With an increase in the concentration of spent wash, there was a decrease in the N and P uptake when plants were irrigated with different levels of digested spent wash. Similarly, in the case of pre-sowing amendment with digested spent wash, the nutrient uptake increased up to 50 m^3 ha^{-1}, but at higher doses it decreased. High nutrient uptake has been reported elsewhere at lower concentrations of spent wash (Patil et al., 2000; Sukanya and Meli, 2004b). The lower uptake of nutrients at higher concentrations of spent wash might be due to the excessive quantities of soluble salts and solid material in digested spent wash.

There was no significant increase in the soil pH with the application of digested distillery spent wash, though the soil EC, percentage organic carbon and total N and P contents increased with increasing doses of spent wash in both types of treatment (irrigation/amendment). A progressive increase in the pH, EC, organic C, and N and P contents of post-harvest soil after the application of increasing doses of distillery spent wash has also been reported elsewhere (Singh and Bahadur, 1997; Zalawadia et al., 1997).

Conclusions

The application of lower doses of digested spent wash to the soil, either as soil amendment or along with irrigation, has a beneficial effect on soil nutrients, thereby increasing the uptake of nutrients by the crop and ultimately resulting in increased crop productivity.

References

Anonymous (1986): *Chemical Oxygen Demand (Dichromate Value) of Polluted and Waste Water* (2nd edition). HMSO, Great Briatin. pp. 8–21. ISBN 0-11-751915-4

Banerjee, A. C., Bajwa, I., Behal, K. K. (2004): Effect of distillery effluent on growth of *Casuarina equisetifolia. J. Indus. Poll. Cont.*, **20**, 199–204.

Bhalerao, V. P., Jadhav, M. B., Power, A. B., Bhoi, P. G. (2004): Effect of pre-sowing application and fertigation of secondary treated biomethanated effluent on soil property and growth of green manuring crop. *Co-operative Sugar*, **36**, 155–159.

Bremner, J. M. (1960): Determination of nitrogen in soil by Kjeldahl method. *J. Agric. Sci.*, **55**, 11–33.

Chandra, R., Kumar, K., Singh, J. (2004): Impact of anaerobically treated and untreated (raw) distillery effluent irrigation on soil microflora, growth, total chlorophyll and protein contents of *Phaseolus aureus* L. *J. Environ. Biol.*, **25**, 381–385.

Deora, A., Kukreja, K., Suneja, S. (2008): Effect of digested distillery effluent on seed germination and plant growth of wheat. *J. Crop. Res.*, **9**, 110–115.

Dudeja, S. S., Chaudhary, P. (2005): Fast chlorophyll fluorescence transient and nitrogen fixing ability of chickpea nodulation variants. *Photosynthetica,* **43**, 253–259.

Gad, A. S., El Sayaad, H. (2010): Biosorption of molasses pigments by sludge agriculture residues and fungal. *J. Appl. Sci. Res.*, **6**, 1722–1729.

John, M. K. (1970): Calorimetric determination of phosphorus in soil and plant material with ascorbic acid. *J. Soil Sci.*, **109**, 214–220.

Juwarkar, A., Dutta, S. A., Juwarkar, A. (1990): Impact of distillery effluent application to land on soil microflora. *J. Environ. Monitor. Assessm.*, **15**, 201–210.

Mahimairaja, S., Bolan, N. S. (2004): Problems and prospects of agricultural use of distillery spent wash in India. In: *Super Soil 2004. Proceedings of the 3rd Australian New Zealand Soils Conf.*, University of Sydney Australia. (www.regional.org.au/au/assoi/supersoil 2004)

Manonmani, K., Chitrarasu, G., Swaminathan, K. (1990): Effect of spentwash on the physico-chemical properties of saline calcareous soil. *J. Poll. Res.*, **9**, 79–82.

Nandy, T., Shastry, S., Kaul, S. N. (2002): Wastewater management in cane molasses distillery involving bioresource recovery. *J. Environ. Manag.*, **65**, 25–38.

Pandey, S. K., Pallavi, T., Gupta, A. K. (2007): Physico-chemical analysis and effect of distillery effluent on seed germination of wheat, pea and lady's finger. *ARPN J. Agric. Biol. Sci.*, **2**, 35–40.

Patil, G. D., Pingat, S. M., Yelwanda, A. J. (2000): Effect of spent wash on soil fertility, uptake, quality and yield of fodder maize. *J. Maharasttra Agric. Uni.*, **25**, 168–170.

Raghukumar, C., Mohandass, C., Kamat, S., Shailaja, M. S. (2004): Simultaneous detoxification and decolorization of molasses spent wash by the immobilized white-rot fungus *Flavadon flavus* isolated from the marine habitat. *J. Enz. Microbial. Tech.*, **35**, 197–202.

Ramana, S., Biswas, A. K., Kundu, S., Saha, J. K., Yadava, R. B. R. (2002a): Effect of distillery effluents on seed germination in some vegetable crops. *J. Bioresour. Technol.*, **82**, 273–275.

Ramana, S., Biswas, A. K., Singh, A. B., Yadava, R. B. R. (2002b): Relative efficacy of different distillery effluents on growth, nitrogen fixation and yield of groundnut. *J. Bioresour. Technol.*, **81**, 117–121.

Sahai, R., Jabeen, S., Saxena, P. K. (1983): Effect of distillery waste on seed germination, seedling growth and pigment content of rice. *Indian J. Ecol.*, **10**, 7–10.

Seyis, I., Subasioglu, T. (2009): Screening of different fungi for decolorization of molasses. *Brazilian J. Microbiol.*, **40**, 61–65.

Singh, D. K., Kumar, D., Singh, U. P. (1985): Studies on pollutional effects of sugar mill and distillery effluent on seed germination and seedling growth of three varieties of rice. *J. Environ. Biol.*, **6**, 31–35.

Singh, Y., Bahadur, R. (1995): Germination of field crop seeds in distillery effluent. *Indian J. Ecol.*, **22**, 82–85.

Singh, Y., Bahadur, R. (1997): Effect of distillery effluent irrigation on maize grain crop and soil fertility. *Indian J. Ecol.*, **24**, 53–59.

Strasser, R. J., Srivastava, A., Tsimilli-Michael, M. (2000): The fluorescence transient as a tool to characterize and screen photosynthetic samples. pp. 445–483. In: Yunus, M., Pathre, U., Mohanty, P. (ed.): *Probing Photosynthesis: Mechanisms, Regulation and Adaptation.* Taylor & Francis, London, New York.

Sukanya, T. S., Meli, S. S. (2004a): Effect of distillery effluent irrigation on growth, yield and quality of maize grown on sandy loam in northern transitional zone of Karnataka. *Karnataka J. Agric. Sci.*, **17**, 405–409.

Sukanya, T. S., Meli, S. S. (2004b): Response of wheat to graded dilution of liquid distillery effluent (spent wash) on plant nutrient contents, nutrient uptake, crop yield and residual soil fertility. *Karnataka J. Agric. Sci.,* **17**, 417–420.

Vincent, J. M. (1970): *A Manual for the Practical Study of Root Nodule Bacteria.* IBP Handbook No. 15. Oxford Blackwell Scientific Publications, Oxford, England. 176 p.

Walkley, A., Black, I. A. (1934): An examination of the Degtzareff method for determination of soil organic matter and proposed modification of the chromic acid titration method. *J. Soil Sci.*, **37**, 29-38.

Zalawadia, N. M., Raman, S., Patil, R. G. (1997): Influence of diluted spent wash of sugar industries application on yield and uptake by sugarcane and changes in soil properties. *J. Indian Soc. Soil Sci.*, **45**, 767–769.

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COMBINING ABILITY AND HETEROSIS STUDIES ACROSS ENVIRONMENTS IN LINSEED (*Linum usitatissimum* L.)

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Combining ability and heterosis were calculated for fourteen lines of linseed in a line × tester mating design using twelve lines and two diverse testers in two different environments. The hybrids and parental lines were raised in a completely randomized block design with three replications to investigate seed and fibre yield and their component traits. Genetic variation was significant for most of the traits over environments. Combining ability studies revealed that the lines KL-221 and LCK-9826 were good general combiners for seed yield and most of its components, whereas LMH-62 and LC-2323 were good general combiners for yield components only. Moreover, KL-221 was also a good general combiner for fibre yield. Similarly, B-509 and Ariane were good general combiners for fibre yield and most of its components. Among the specific cross combinations, B-509 × Flak-1 was outstanding for seed yield per plant and B-509 × KL-187 and LC-2323 × LCK-9826 for fibre yield per plant, with high SCA effects. In general, the hybrids excelled their respective parents and the standard checks for most of the characters studied. Based on the comparison of mean performance, SCA effects and the extent of heterosis, the hybrids LC-2323 × LCK-9826 and B-509 × KL-221 appeared to be the most promising for both seed and fibre yield. Other promising combinations were LC-2323 × KL-210 and B-509 × Ariane for seed and fibre yield, respectively. The superiority of LC-2323, LCK-9826, KL-221, B-509 and Ariane as good general combiners was further confirmed by the involvement of these parents in the desirable cross combinations.

Key words: combining ability, fibre yield, GCA, heterosis, linseed, *Linum usitatissimum*, SCA, seed yield

Introduction

Oilseeds command second major agricultural crop status in India next to food grains in terms of both production and value. In India, linseed (*Linum usitatissimum* L.) is one of the most important non-edible oilseed crops, cultivated for seed as well as fibre. The fibre is used for the manufacture of linen and the seeds as oil and cake. The oil content in the seed generally varies from 33–45% (Gill, 1987a). Although linseed oil is primarily an industrial product, in

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India 25% of the total linseed oil produced is used for domestic purposes (Anonymous, 2004). The contribution of India to global linseed area and production is 21.2 and 8.2%, respectively (Anonymous, 2008), but the productivity of linseed in India is very low (376 kg/ha) in comparison to the world average of 778 kg/ha, because it is normally grown under rainfed conditions by resource-poor farmers, and the non-availability of high potential cultivars also lowers the productivity of this oilseed (Rai et al., 2002). This crop is gaining momentum because of the great demand for its seed and fibre for manufacturing items of industrial significance (Yadav and Srivastava, 2002).

Linseed being an autogamous crop, genetic improvement has been carried out through conventional breeding methods, i.e. selection and hybridization. Selection is only effective if variability is present in the crop or population to be improved. Variability is created either through hybridization, through mutation breeding or through polyploidy. The success of hybridization programmes depends on the ability of the parents involved to yield desirable segregants/recombinants (Hallauer and Miranda, 1981). The ability of the parents to combine well depends on complex interactions between the genes, which cannot be judged by mere yield performance and the adaptation of the parents. Parents with good *per se* performance may not necessarily produce desirable progenies when used in hybridization (Allard, 1960). Moreover, a particular cross producing transgressive segregants in autogamous crops like linseed would depend upon the precise estimates of various components, namely additive, dominance, non-allelic interactions, linkage between polygenes and gene dispersion in the parents of the cross, contributing to heterosis (Jinks, 1983). Combining ability analysis is an important tool for the selection of desirable parents, together with information on the nature and magnitude of gene effects controlling quantitative traits of economic importance. Moreover, such information is more reliable when drawn from various environments. The present investigation was therefore undertaken to generate information on combining ability effects for yield and its component traits and for oil content in linseed grown in varying environments.

Materials and methods

The present investigation was carried out at two locations, Department of Plant Breeding and Genetics, CSK HPKV, Palampur, and Wheat and Rice Research Centre (WRRC), Malan, Himachal Pradesh, India.

Palampur: The experimental farm is situated at 32°8′ N latitude and 76°3′ E longitude at an elevation of 1290.8 m above mean sea level. Agro-climatically, the location represents the mid-hill zone of Himachal Pradesh (Zone II) and is characterized by a humid sub-temperate climate with high rainfall (2500 mm). The soil is acidic in nature, with a pH ranging from 5.0 to 5.6.

Malan: The experimental farm is situated at an elevation of 950 m above mean sea level at 32°07′ N latitude and 76°23′ E longitude representing sub-humid mid-hill conditions having high rainfall. The soil of the experimental area is a clay loam with a pH ranging from 5.8 to 6.0.

Experimental materials

The experimental materials comprised 14 genotypes of linseed. Of these, two genotypes, KL-210 (seed type) and B-509 (dual purpose) were used as standard checks I and II, respectively, the former for seed yield and the latter for dual-purpose, i.e. both seed and fibre yield (Table 1). Two agronomically superior but genetically diverse lines, B-509 (dual purpose) and LC-2323 (seed type) were used as testers. The parentage/source of the genotypes used in the study is given in Table 1.

Table 1
Parentage/source of genotypes

Genotype	Parentage/ Source	Category on the basis of use
Ariane*	Exotic line from Belgium	Fibre flax
Belinka*	Exotic line from Belgium	Fibre flax
Aoyagi*	Selection from exotic Ayogi	Fibre flax
Flak-1*	Selection from exotic flax line	Fibre flax
LMH-62*	(EC-41628 × EC-77959) × (DPL-20 × Neelum)	Dual-purpose
LCK-9826*	LCK-88062 × KL-168	Dual-purpose
KL-187*	K-2 × TLP-1	Seed flax
KL-178	LC-36 × LC-255	Dual-purpose
KL-233*	Flax purple × Gaurav	Dual-purpose
Janaki*	New River × LC-216	Seed flax
KL-221*	89D-2B/ 5 × SPS 47/ 7-10-3	Seed flax
KL-210*	Flak-1 × SPS 47/ 7-10-3	Seed flax
B-509 **	Birsa Agricultural University (Bihar)	Dual-purpose
LC-2323**	Punjab Agricultural University (Punjab)	Seed flax

*: Line; **: Tester; Fibre flax: mainly used for fibre extraction from the stem; Seed flax: used for oil and cake extraction from the seeds; Dual-purpose flax: used for both fibre and oil extraction

Crossing plan

The crosses followed the mating design proposed by Kempthorne (1957). In the 2002–03 season, the above-mentioned twelve linseed lines were crossed to two testers, B-509 and LC-2323, to generate 24 hybrids. The crosses were repeated during summer 2003 at the Mountain Agricultural Research and Extension Centre, Sangla (Kinnaur), located at 2680 m amsl 31°25' N latitude and 78°15' E longitude, to increase the seed of the 24 progenies.

Experimental design and layout

All the 24 hybrids along with the 12 lines and two testers were sown in a completely randomized block design with three replications during the 2003–04 season at Palampur (E_1) on 16th November, 2003 and Malan (E_2) on 2nd December, 2003. Each hybrid and parent was raised in three rows, 1.5 m long with row-to-row and plant-to-plant spacings of 30 cm and 5 cm, respectively.

The experimental fields were well prepared and the recommended dose of fertilizer (50 kg N, 40 kg P_2O_5 and 20 kg K_2O per hectare) was applied. Half the dose of nitrogen and the full dose of phosphorus and potash were applied as basal and the remaining half nitrogen was top dressed two months after sowing. Irrigation was given whenever required and regular weeding was done to keep the trial free from weeds.

Recording of observations

Ten competitive plants were tagged randomly from each genotype in each replication to record observations for days to first flower, height at first flowering (cm), days to 50% flowering, days to 75% maturity, plant height at maturity (height from ground level to the top of the plant measured in cm), technical height at maturity (height from ground level to the point where primary branching starts, measured in cm), primary branches per plant, capsules per plant, tillers per plant, seeds per capsule, seed yield per plant (g), thousand-seed weight (g), straw yield per plant (g), biological yield per plant (g), harvest index (%), retted straw yield per plant (g), fibre yield per plant (g) and oil content (%). Harvest index (%) was calculated as:

$$\frac{\text{Seed yield per plant (g)}}{\text{Biological yield per plant (g)}} \times 100$$

Statistical analysis

The mean values of all observations for each location were analysed separately and then their combined data were analysed. Analysis of variance for a randomized block design was done using the model suggested by Panse and Sukhatme (1984). The line × tester analysis, combining ability analysis and estimation of GCA and SCA were carried out using the methods given by Kempthorne (1957). The statistical analysis was done using SPAR software developed by the Indian Agricultural Statistical Research Institute, New Delhi.

Results

The analysis of variance for combining ability revealed significant differences between the hybrids for all the characters studied over all the environments, except for capsules per plant (Table 2). The partitioning of mean squares for hybrids revealed that the mean squares for lines and testers were also significant for most of the traits, indicating significant differences between the lines and testers for their general combining ability effects. The mean squares for environment were also significant, implying the varying nature of the environments. The non-significance of the mean squares for line × environment, tester × environment and line × tester × environment for the majority of the traits suggested that the GCA effects of the parents and the SCA effects of the hybrids were not influenced by the environments. These results are in close proximity with the findings of Kumar et al. (2000), Bhateria et al. (2001) and Sood (2004).

General combining ability effects

In general, no single parental line proved to be a good general combiner for all the traits studied (Table 3). The line KL-221 was a good general combiner for eight traits (higher seed yield per plant, capsules per plant, 1000-seed weight, primary branches per plant, harvest index and fibre yield per plant, and decreasing days to first flower and days to 50% flowering), Ariane for seven traits (higher fibre yield per plant, retted straw weight per plant, biological yield per plant, straw yield per plant, technical height, plant height and height at first flowering), LMH-62 for three traits (decreasing days to first flower, days to 50% flowering and days to 75% maturity) and Janaki for increasing oil content and 1000-seed weight (Tables 3and 4).

The tester LC-2323 was a good general combiner for high harvest index, tillers per plant, 1000-seed weight and oil content and decreasing days to first flower and days to 50% flowering, whereas B-509 was a good general combiner for increasing height at first flowering, plant height, technical height, straw yield per plant, retted straw weight per plant, fibre yield per plant and days to 75% maturity (Tables 3 and 4).

Table 2
Combined analysis of variance for combining ability over environments for various biometrical traits in linseed

S. of V.	Environ.	Repl.	Hybrid	Lines	Tester	L × T	L × E	T × E	L×T×E	Error
Trait d.f.	1	4	23	11	1	11	11	1	11	92
DFF	4646.58*	36.33*	40.61*	52.25*	300.36*	5.35	5.48	44.53*	3.94	3.78
HF (cm)	469.44*	33.92	855.38*	722.92*	10902.83*	74.44	90.64*	51.36	40.94	44.95
DF	5112.25*	24.46*	42.24*	46.45*	386.81*	6.71*	3.07	66.64*	2.58	2.94
DM	4011.03*	48.72*	27.08*	36.39*	46.81*	16.02	22.48*	7.86	8.95	10.46
PH (cm)	233.91*	100.79*	931.10*	635.80*	14142.58*	25.36	24.09	7.42	21.38	26.04
TH (cm)	182.00*	31.91*	475.09*	336.91*	7065.54*	14.15	15.92	6.89	13.07	8.62
PB	242.58*	22.99*	5.16*	8.25*	6.89	1.91	7.88	23.77*	3.94	3.80
CP	27863.97*	1456.35*	320.01*	446.98*	0.06	222.14	223.81	0.00	166.16	230.33
TP	42.52*	1.42*	1.09*	0.75	14.79*	0.18	0.41	1.24	0.51	0.47
SC	26.09*	0.58	2.31*	1.67*	4.52*	2.75*	0.28	0.02	0.78	0.46
SY (g)	23.49*	2.36*	1.08*	1.62*	0.74	0.56	0.37	0.04	0.54	0.62
TSW (g)	0.85*	0.11	3.52*	4.69*	25.59*	0.34*	0.16	0.24	0.12	0.10
StY (g)	12.87*	3.03*	2.42*	1.90*	23.96*	0.99	1.09	0.06	0.57	0.79
BY (g)	24.16*	6.75*	4.92*	4.69*	18.57*	3.91*	0.33	0.12	0.59	2.05
HI (%)	830.42*	103.79*	311.09*	351.52*	2881.89*	36.95	19.04	7.02	35.12	31.75
RStY (g)	13.69*	2.09*	1.76*	1.21	19.21*	0.74	0.84	0.03	0.57	0.69
FY (g)	9.25*	0.42	0.39*	0.44	1.76*	0.22	0.27	0.00	0.29	0.26
OC (%)	0.13	2.57*	9.43*	10.06*	97.74*	0.76	0.65	1.29	0.75	0.94

S. of V.: source of variation; Environ. Environment; Repl.: replication; L × T: Line × Tester; L × E: Line × environment; T × E: Tester × environment; L × T × E: Line × tester × environment; * Significant at $P \leq 0.05$; DFF: Days to first flower; HF: Height at first flowering; DF: Days to 50% flowering; DM: Days to 75% maturity; PH: Plant height at maturity; TH: Technical height at maturity; PB: Primary branches per plant; CP: Capsules per plant; TP: Tillers per plant; SC: Seeds per capsule; SY: Seed yield per plant; TSW: Thousand-seed weight; StY: Straw yield per plant; BY: Biological yield per plant; HI: Harvest index; RStY: Retted straw yield per plant; FY: Fibre yield per plant; OC: Oil content

Specific combining ability effects

Any generalization from the results for specific combining ability is difficult as no individual cross combination revealed significant desirable SCA effects for all the characters (Table 5). However, from the economic point of view, seed and fibre yield are the most important traits. Therefore, greater emphasis needs to be placed on these characters. The hybrid B-509 × Flak-1 was exceptionally good, with desirable SCA effects for seed yield per plant, seeds per capsule, biological yield per plant and technical height. Similarly, B-509 × KL-187 and LC-2323 × LCK-9826 were desirable for fibre yield per plant (Table 5). The parents involved in the hybrid B-509 × Flak-1 were poor performers for seed yield per plant, suggesting that poor × poor parental combinations could also be of use in the production of hybrids, due to the complementation of favourable genes.

Table 3

General combining ability (GCA) effects of parents for different biometrical traits over environments in linseed

Trait	DFF	HF (cm)	DF	DM	PH (cm)	TH (cm)	PB	CP	TP	SC	SY (g)	TSW (g)	StY (g)	BY (g)	HI (%)	RStY (g)	FY (g)	OC (%)
Line																		
LMH-62	–3.35*	–5.34*	–3.47*	–2.06*	–6.95*	–5.66*	0.06	–0.48	0.37*	0.15	0.01	–0.11	–0.03	–0.14	1.15	–0.03	–0.04	–0.14
Flak-1	–1.43*	–2.22	–0.64	1.44*	–0.8	0.68	–0.71	–4.29	–0.17	0.03	–0.16	0.14	–0.11	–0.14	–0.22	–0.07	–0.17	0.01
Belinka	2.32*	15.03*	2.53*	–3.14*	10.94*	9.25*	–0.31	–3.28	–0.08	–1.08*	–0.61*	–0.96*	–0.01	–0.39	–10.05*	0.01	–0.06	–1.21*
Aoyagi	1.74*	3.28*	2.03*	0.94	9.70*	4.75*	0.34	8.86*	0.03	0.31*	0.22	–0.36*	0.56*	0.69*	–1.08	0.48*	0.24*	–0.35
KL-221	–2.18*	–8.22*	–1.64*	–1.06	–6.95*	–4.19*	2.08*	12.81*	0.33*	0.03	0.62*	0.28*	0.21	0.93*	4.62*	0.15	0.29*	0.4
KL-178	0.4	–2.84	–0.39	3.28*	–3.09*	–2.33*	–0.06	0.27	–0.05	–0.13	0.02	0.66*	–0.21	–0.15	1.47	–0.1	–0.06	0
KL-187	–0.76	–2.05	–0.22	1.28	–1.34	–2.14*	–1.01*	–5.19	–0.37*	0.02	–0.06	0.44*	–0.41*	–0.11	3.40*	–0.38*	–0.06	0.4
KL-210	–0.35	–2.38	–0.47	0.28	–4.01*	–2.88*	0.18	2.19	0.18	0.30*	0.35*	0.03	–0.07	0.16	4.97*	–0.07	–0.1	0.1
Janaki	0.15	–1.8	–0.31	–0.47	–3.95*	–1.49*	–1.04*	–7.81*	–0.37*	0.22	–0.25	0.40*	–0.56*	–0.71*	0.68	–0.42*	–0.18	1.00*
Ariane	4.15*	15.66*	3.86*	–1.39	14.04*	10.44*	0.49	0.36	0.1	–0.13	–0.44*	–1.35*	0.82*	0.83*	–11.65*	0.65*	0.29*	–2.10*
LCK-9826	–1.60*	–7.47*	–1.22*	0.11	–2.54*	–3.69*	0.28	2.69	0.22	0.25	0.48*	0.73*	0.18	0.25	3.62*	0.06	0.14	1.15*
KL-233	0.90*	–1.67	–0.06	0.78	–5.04*	–2.74*	–0.31	–6.13	–0.2	0.03	–0.19	0.1	–0.36	–1.21*	3.12*	–0.28	–0.28*	0.73*
S.E. ± (gi)	0.43	1.47	0.38	0.71	1.12	0.64	0.43	3.33	0.15	0.15	0.17	0.13	0.2	0.31	1.24	0.18	0.11	0.21
S.E. ± (gi-gj)	1.12	3.87	0.99	1.87	2.68	1.69	1.13	8.76	0.39	0.39	0.45	0.19	0.51	0.83	3.25	0.48	0.29	0.56
Tester																		
B-509	1.44*	8.70*	1.64*	–0.57*	9.91*	7.00*	–0.22	–0.02	–0.32*	0.18*	–0.07	–0.42*	0.41*	0.36*	–4.47*	0.37*	0.11*	–0.82*
LC-2323	–1.44*	–8.70*	–1.64*	0.57*	–9.91*	–7.00*	0.22	0.02	0.32*	–0.18*	0.07	0.42*	–0.41*	–0.36*	4.47*	–0.37*	–0.11*	0.82*
S.E. ± (gi)	0.13	0.44	0.11	0.21	0.34	0.19	0.13	1.01	0.05	0.04	0.05	0.05	0.06	0.09	0.37	0.06	0.03	0.06
S.E. ± (gi-gj)	0.46	1.58	0.4	0.76	1.2	0.69	0.46	3.58	0.16	0.16	0.19	0.08	0.21	0.34	1.32	0.19	0.12	0.23

* Significant at $P \leq 0.05$, DFF: Days to first flower; HF: Height at first flowering; DF: Days to 50% flowering; DM: Days to 75% maturity; PH: Plant height at maturity; TH: Technical height at maturity; PB: Primary branches per plant; CP: Capsules per plant; TP: Tillers per plant; SC: Seeds per capsule; SY: Seed yield per plant; TSW: Thousand-seed weight; StY: Straw yield per plant; BY: Biological yield per plant; HI: Harvest index; RStY: Retted straw yield per plant; FY: Fibre yield per plant; OC: Oil content

Table 4

List of good general combiners (lines and testers) for different traits over two environments

Traits	Good general combiners	
Days to first flower	LMH-62, Flak-1, KL-221, LCK-9826, LC-2323	
Height at first flowering	Seed flax	Fibre flax
	KL-221, LCK-9826, LMH-62, LC-2323	Belinka, Ariane, B-509
Days to 50% flowering	LMH-62, KL-221, LCK-9826, LC-2323	
Days to 75% maturity	LMH-62, Belinka, B-509	
Plant height (cm)	Seed flax	Fibre flax
	LMH-62, KL-221, KL-233, KL-210, Janaki, LC-2323	Belinka, Aoyagi, Ariane, B-509
Technical height (cm)	Seed flax	Fibre flax
	LMH-62, KL-221, LCK-9826, KL-210, KL-233, KL-178, LC-2323	Belinka, Aoyagi, Ariane, B-509
Primary branches per plant	KL-221	
Capsules per plant	KL-221	
Tillers per plant	LC-2323	
Seeds per capsule	-	
Seed yield per plant (g)	KL-221, LCK-9826	
1000-seed weight (g)	KL-221, KL-178, KL-187, Janaki, LCK-9826, LC-2323	
Straw yield per plant (g)	Seed flax	Fibre flax
	KL-187, LC-2323	Ariane, B-509
Biological yield per plant (g)	Ariane	
Harvest index (%)	KL-221, KL-187, KL-210, LCK-9826, KL233, LC-2323	
Retted straw weight per plant (g)	Aoyagi, Ariane, B-509	
Fibre yield per plant (g)	KL-221, Ariane, B-509	
Oil content (%)	Janaki, LCK-9826, KL-233, LC-2323	

Table 5
Estimates of specific combining ability (SCA) effects for different traits pooled over environments in linseed

Trait Cross	DFF	HF (cm)	DF	DM	PH (cm)	TH (cm)	PB	CP	TP	SC	SY (g)	TSW (g)	StY (g)	BY (g)	HI (%)	RStY (g)	FY (g)	OC (%)
B-509×LMH-62	–0.03	0.97	0.36	1.65*	1.8	–0.44	0.89*	9.33*	–0.1	–0.91*	0.21	0.25	0.53*	0.93*	–2.66*	0.39*	0.21	–0.03
B-509×Flak-1	0.39	2.84	0.19	–2.01*	2.28*	1.74*	0.29	5.12	0.07	0.41*	0.48*	0.16	0.38	0.87*	2.21	0.40*	0.08	0.22
B-509×Belinka	–0.53	–2.58	–1.14*	1.4	–2.15	–2.35*	0.09	–3.93	0.09	0.52*	–0.27	0.02	–0.33	–1.05*	–0.79	–0.37*	–0.15	0.36
B-509×Aoyagi	0.22	2.51	0.36	0.49	0.61	0.14	0.13	2.08	–0.1	0.41*	–0.09	–0.35	0.18	0.05	–0.59	0.06	–0.11	–0.18
B-509×KL-221	0.14	–0.66	–0.14	–1.01	–0.16	0.36	–0.4	–1.92	–0.1	0.17	0.07	0.04	–0.06	0.07	0.64	0.01	0.05	–0.14
B-509×KL-178	0.56	2.97*	0.44	–0.68	0.47	0.53	–0.16	0.82	–0.2	–0.49*	0	0.04	–0.1	–0.43	1.62	0.08	0	–0.18
B-509×KL-187	0.22	2.59	0.61	0.15	0.21	–0.95	–0.01	1.75	0.04	–0.18	0.09	–0.18	0.13	0.6	–1.71	0.13	0.22*	0.09
B-509×KL-210	0.14	–1.74	0.03	–0.51	–1.24	–0.49	0.27	–0.37	0.19	–0.09	–0.17	0.15	–0.09	–0.17	–1.81	–0.13	0.02	–0.08
B-509×Janaki	0.81	0.76	0.03	1.07	–0.51	0.2	–0.28	–0.53	0.04	0.06	0.14	–0.02	–0.07	–0.05	2.71*	–0.01	0.01	0.07
B-509×Ariane	–1.19*	–3.37*	–0.64	0.49	–2.62*	–0.74	–0.41	–3.4	–0.1	0.74*	–0.11	–0.09	–0.47*	–0.38	1.54	–0.36*	–0.08	0.17
B-509×LCK-9826	0.56	–3.91*	1.28*	–1.51*	0.81	0.8	–0.56	–6.83*	–0.1	–0.24	–0.28	–0.13	–0.24	–0.39	0.24	–0.23	–0.24*	–0.57*
B-509×KL-233	–1.28*	–0.37	–1.39*	0.49	0.51	1.21	0.19	–2.12	0.2	–0.39*	–0.08	0.1	0.13	–0.07	–1.39	0.03	–0.01	0.28
LC-2323×LMH-62	0.03	–0.97	–0.36	–1.65*	–1.8	0.44	–0.89*	–9.33*	0.06	0.91*	–0.21	–0.25	–0.53*	–0.93*	2.66*	–0.39*	–0.21	0.03
LC-2323×Flak-1	–0.39	–2.84	–0.19	2.01*	–2.28*	–1.74*	–0.29	–5.12	–0.1	–0.41*	–0.48*	–0.16	–0.38	–0.87*	–2.21	–0.40*	–0.08	–0.22
LC-2323×Belinka	0.53	2.58	1.14*	–1.4	2.15	2.35*	–0.09	3.93	–0.1	–0.52*	0.27	–0.02	0.33	1.05*	0.79	0.37*	0.15	–0.36
LC-2323×Aoyagi	–0.22	–2.51	–0.36	–0.49	–0.61	–0.14	–0.13	–2.08	0.1	–0.41*	0.09	0.35	–0.18	–0.05	0.59	–0.06	0.11	0.18
LC-2323×KL-221	–0.14	0.66	0.14	1.01	0.16	–0.36	0.4	1.92	0.1	–0.17	–0.07	–0.04	0.06	–0.07	–0.64	–0.01	–0.05	0.14
LC-2323×KL-178	–0.56	–2.97*	–0.44	0.68	–0.47	–0.53	0.16	–0.82	0.18	0.49*	0	–0.04	0.1	0.43	–1.62	–0.08	0	0.18
LC-2323×KL-187	–0.22	–2.59	–0.61	–0.15	–0.21	0.95	0.01	–1.75	–0	0.18	–0.09	0.18	–0.13	–0.6	1.71	–0.13	–0.22*	–0.09
LC-2323×KL-210	–0.14	1.74	–0.03	0.51	1.24	0.49	–0.27	0.37	–0.2	0.09	0.17	–0.15	0.09	0.17	1.81	0.13	–0.02	0.08
LC-2323×Janaki	–0.81	–0.76	–0.03	–1.07	0.51	–0.2	0.28	0.53	–0	–0.06	–0.14	0.02	0.07	0.05	–2.71*	0.01	–0.01	–0.07
LC-2323×Ariane	1.19*	3.37*	0.64	–0.49	2.62*	0.74	0.41	3.4	0.13	–0.74*	0.11	0.09	0.47*	0.38	–1.54	0.36*	0.08	–0.17
LC-2323×LCK-9826	–0.56	3.91*	–1.28*	1.51*	–0.81	–0.8	0.56	6.83*	0.05	0.24	0.28	0.13	0.24	0.39	–0.24	0.23	0.24*	0.57*
LC-2323×KL-233	1.28*	0.37	1.39*	–0.49	–0.51	–1.21	–0.19	2.12	–0.2	0.39*	0.08	–0.1	–0.13	0.07	1.39	–0.03	0.01	–0.28
SE±(Sij)	0.43	1.47	0.38	0.71	1.12	0.64	0.43	3.33	0.15	0.15	0.17	0.19	0.2	0.31	1.24	0.18	0.11	0.21
SE±(Sij-Sik)	1.59	5.47	1.4	2.64	4.17	2.39	1.59	12.39	0.56	0.55	0.64	0.26	0.73	1.17	4.6	0.68	0.42	0.79

* Significant at $P \leq 0.05$; DFF: Days to first flower; HF: Height at first flowering; DF: Days to 50% flowering; DM: Days to 75% maturity; PH: Plant height at maturity; TH: Technical height at maturity; PB: Primary branches per plant; CP: Capsules per plant; TP: Tillers per plant; SC: Seeds per capsule; SY: Seed yield per plant; TSW: Thousand-seed weight; StY: Straw yield per plant; BY: Biological yield per plant; HI: Harvest index; RStY: Retted straw yield per plant; FY: Fibre yield per plant; OC: Oil content

The cross combinations LC-2323 × LMH-62 (involving poor × good combiners for days to 75% maturity, poor × average for seeds per capsule, good × average for straw yield per plant and good × average for harvest index), B-509 × Ariane (days to first flower: poor × poor, plant height: poor × poor, seeds per capsule: average × poor and straw yield per plant: poor × poor), B-509 × Belinka (days to 50% flowering: poor × poor, technical height: poor × poor and seeds per capsule: average × poor), B-509 × KL-233 (days to first flower: poor × poor), B-509 × LCK-9826 (height at first flowering: poor × good), LC-2323 × Flak-1 (technical height: good × poor), B-509 × Aoyagi (average × average), LC-2323 × KL-178 (poor × poor) and LC-2323 × KL-233 (poor × average) for seeds per capsule, and B-509 × Janaki (harvest index: poor × average) were desirable for seed type linseed genotypes. Other promising hybrids exhibited desirable SCA effects for both seed and fibre traits: B-509 × LMH-62 (capsules per plant: poor × poor, biological yield per plant: average × poor, straw yield: good × poor and retted straw weight per plant: good × poor); LC-2323 × Belinka (technical height: poor × good, biological yield per plant: poor × poor and retted straw weight per plant: poor × average) (Table 5). Good × good GCA combinations could be attributed to additive or additive × additive types of gene actions, which are fixable in nature. Crosses which involved one parent with good and one with

poor GCA effects could throw up transgressive segregants if the additive effect of one parent and the complementary epistatic effects act in the same direction and maximize the desirable plant attributes. Cross combinations observed to have high SCA effects involved all possible combinations of parents with poor, average and good general combining ability. This indicated that in general GCA had no bearing on the SCA effects of the crosses. This finding is in agreement with those of Thakur and Rana (1987), Singh and Srivastava (1987), Thakur and Bhateria (1991), Mahto and Rahman (1998), Pathania (1999), Kumar et al. (2000), Bhateria et al. (2001) and Sood (2004), who also reported the involvement of good × good, good × average, good × poor, average × average, average × poor and poor × poor combiners in hybrids revealing significant SCA effects in linseed. In contrast to these results, Badwal and Gupta (1970) reported that crosses showing high SCA effects involved at least one parent with good general combining ability, whereas Pillai et al. (1995) reported that high × high general combiners showed high SCA effects.

Heterosis

A large number of cross-pollinating species show varying degrees of heterosis, but its magnitude is relatively smaller in self-pollinated crops. Mather (1973) attributed this to the type of genic balance that a crop has acquired during the process of evolution. However, hybrid vigour has also been reported in self-pollinated crops (Tandon, 1980; Gill, 1987b) and is being commercially utilized in crops like rice, etc. The scope for the exploitation of hybrid vigour depends upon the direction and magnitude of heterosis. The interest of breeders lies in hybrids superior to the standard checks. In the present study no single cross combination exhibited significant desirable heterosis for all the characters simultaneously. The cross LC-2323 × LCK-9826, which expressed the maximum extent of economic heterosis for seed yield per plant, also exhibited desirable heterosis for 1000-seed weight, tillers per plant, capsules per plant, days to 50% flowering, primary branches per plant and biological yield per plant over both the standard checks, and desirable heterosis for retted straw weight per plant and fibre yield per plant over standard check I and for days to first flower, seeds per capsule, harvest index and oil content over standard check II (Tables 6 and 7). Two more cross combinations, B-509 × KL-221 and LC-2323 × KL-210, exhibited desirable heterosis for seed yield per plant over both the standard checks. Besides revealing high heterosis for seed yield per plant, cross combination B-509 × KL-221 also expressed desirable heterosis for days to 75% maturity, capsules per plant, biological yield per plant, retted straw weight per plant and fibre yield per plant over standard check I and for days to first flower, days to 50% flowering, primary branches per plant, capsules per plant, seeds per capsule, 1000-seed weight, biological yield per plant, harvest index, fibre yield per plant and oil content over standard check II. The other crosses exhibiting high standard heterosis were LC-2323 × KL-221 (primary branches per plant,

Table 6

Standard heterosis for seed yield and its component traits over environments in linseed

F_1	DF	DM	PH	PB	CP	SC	TP	BY	SY	HI	TSW	OC
B-509 × LMH-62	–1.05	–2.69*	39.82*	22.83	71.97*	15.15*	8.49	79.76*	32.68	–27.13*	0	–9.27*
B-509 × Flak-1	1.35	–2.80*	53.55*	4.13	45.58	–0.61	–10.85	78.00*	39.22	–19.51*	1.54	–8.27*
B-509 × Belinka	3.01*	–3.53*	68.71*	6.88	19.19	–12.73*	–5.66	14.37	–38.56	–47.42*	–16.92*	–10.99*
B-509 × Aoyagi	3.92*	–1.56	71.58*	16.23	78.84*	2.79	–9.43	78.29*	27.45	–27.49*	–13.85*	–10.14*
B-509 × KL-221	0.15	–3.74*	35.73*	33.01*	78.67*	–3.39	5.19	85.92*	64.05*	–12.39	3.08	–8.19*
B-509 × KL-178	1.8	–0.83	45.06*	6.88	46.47	–13.58*	–16.51	39.58*	19.61	–17.12*	7.69*	–9.27*
B-509 × KL-187	2.11*	–1.56	48.13*	–4.13	31.55	–7.88*	–21.23	70.67*	20.92	–20.16*	1.54	–7.62*
B-509 × KL-210	1.35	–2.59*	39.61*	15.95	48.87*	–3.39	11.79	56.30*	30.72	–16.97*	0	–8.77*
B-509 × Janaki	1.51	–2.08	41.23*	–8.25	15.45	–2.67	–21.23	34.31	11.11	–16.47*	3.08	–6.19*
B-509 × Ariane	4.66*	–3.01*	74.16*	11	32.86	1.45	–7.07	69.79*	–16.99	–45.83*	–24.62*	–13.69*
B-509 × LCK-9826	1.8	–3.32*	46.89*	5.91	29.25	–5.82	2.36	52.79*	31.37	–15.44*	6.15*	–7.39*
B-509 × KL-233	0.45	–1.66	41.08*	8.25	15.77	–10.30*	–5.66	19.06	1.31	–20.10*	0	–6.32*
LC-2323 × LMH-62	–4.66*	–4.05*	–8.75	4.54	10.75	2.67	44.81*	4.11	14.38	3.92	4.62	–4.99*
LC-2323 × Flak-1	–1.95*	0.42	3.0	2.2	12.06	–14.79*	13.21	5.87	–14.38	–9.66	9.23*	–5.25*
LC-2323 × Belinka	2.11*	–4.57*	36.52*	10.45	45.15	–29.69*	16.51	54.84*	5.23	–24.52*	–4.62	–8.64*
LC-2323 × Aoyagi	0.31	–1.45	28.22*	18.71	30.43*	–11.52*	30.66*	54.25*	47.71	–5.44	10.77*	–5.15*
LC-2323 × KL-221	–2.55*	–1.76	–4.71	49.93*	91.36*	–11.88*	44.81*	60.70*	64.05*	4.26	13.85*	–3.37*
LC-2323 × KL-178	–1.95*	0.73	1.99	17.33	41.21	–5.82	30.66*	43.69	29.41	–4.72	20.00*	–4.27*
LC-2323 × KL-187	–1.95*	–1.04	6.18	2.2	20.18	–7.88*	5.19	14.96	18.3	6.74	20.00*	–3.95*
LC-2323 × KL-210	–1.65	–1.25	3.63	14.58	51.39*	–5.45	24.06	44.87*	62.09*	10.35	7.69*	–4.27*
LC-2323 × Janaki	–1.49	–2.69*	2.24	5.5	19.06	–8.24*	5.19	16.13	2.61	–8.79	16.92*	–2.39
LC-2323 × Ariane	2.86*	–2.91*	43.92*	28.34*	55.34*	–20.85*	35.38*	70.97*	5.88	–33.06*	–9.23*	–10.39*
LC-2323 × LCK-9826	–3.46*	–0.73	2.45	27.51*	74.27*	–4.24	36.79*	54.25*	77.78*	2.96	23.08*	–0.45
LC-2323 × KL-233	0	–1.56	–2.12	9.08	29.81	–5.09	5.19	2.05	20.26	5.44	10.77*	–3.62*
S.E.± (d)	1.09	1.81	2.88	0.94	7.19	0.31	0.32	0.67	0.37	3.01	0.019	0.59

* Significant at $P \leq 0.05$ Heterosis over standard check KL-210 (seed type) over environments; DF: Days to 50% flowering; DM: Days to 75% maturity; PH: Plant height at maturity; PB: Primary branches per plant; CP: Capsules per plant; TP: Tillers per plant; SC: Seeds per capsule; SY: Seed yield per plant; TSW: Thousand-seed weight; BY: Biological yield per plant; HI: Harvest index; OC: Oil content

Table 7
Standard heterosis for fibre yield and its components over environments in linseed

F_1s	DFF	HF	TH	StY	RStY	FY
B-509 × LMH-62	–4.88*	–25.58*	–37.68*	24.58	22.47	36.58
B-509 × Flak-1	–2.74*	–18.64*	–23.46*	16.84	20.97	15.45
B-509 × Belinka	–0.15	–2.19	–15.98*	–3.7	–4.49	5.69
B-509 × Aoyagi	0	–11.46*	–19.33*	32.32*	29.21*	32.52
B-509 × KL-221	–3.65*	–31.83*	–33.89*	12.79	14.98	50.41*
B-509 × KL-178	–0.92	–19.33*	–30.50*	–3.03	7.87	17.89
B-509 × KL-187	–2.29*	–18.75*	–32.67*	–1.68	–0.75	35.77
B-509 × KL-210	–1.98	–25.24*	–33.11*	2.02	1.12	16.26
B-509 × Janaki	–0.92	–16.78*	–29.67*	–13.47	–7.12	8.13
B-509 × Ariane	0.91	–2.43	–11.30*	19.53	19.85	39.84*
B-509 × LCK-9826	–2.74*	–35.31*	–32.34*	5.39	2.25	13.82
B-509 × KL-233	–2.13*	–22.33*	–30.05*	0	–0.75	–0.81
LC-2323 × LMH-62	–7.46*	–52.43*	–59.59*	–38.72*	–34.36*	–16.26
LC-2323 × Flak-1	–6.09*	–50.69*	–52.64*	–36.70*	–36.33*	–16.26
LC-2323 × Belinka	–1.83	–19.21*	–31.52*	–9.09	–4.49	12.19
LC-2323 × Aoyagi	–3.05*	–42.59*	–43.19*	–6.73	–2.62	32.52
LC-2323 × KL-221	–6.55*	–54.17*	–58.48*	–10.77	–13.11	24.39
LC-2323 × KL-178	–4.57*	–51.74*	–55.64*	–23.57	–25.09	0
LC-2323 × KL-187	–5.33*	–50.11*	–52.87*	–38.38*	–37.45*	–18.69
LC-2323 × KL-210	–4.88*	–44.56*	–54.87*	–19.19	–16.48	–4.88
LC-2323 × Janaki	–5.03*	–47.22*	–53.71*	–36.70*	–33.71*	–10.57
LC-2323 × Ariane	0.46	–17.25*	–32.22*	23.57	19.1	34.15
LC-2323 × LCK-9826	–6.40*	–48.61*	–58.38*	–5.72	–7.49	35.77
LC-2323 × KL-233	–2.43*	–45.49*	–57.48*	–36.03*	–29.96*	–17.07
S.E.± (d)	1.17	3.68	1.6	0.42	0.39	0.24

* Significant at $P \leq 0.05$ Heterosis over standard check B-509 (flax type); DFF: Days to first flower; HF: Height at first flowering; TH: Technical height at maturity; StY: Straw yield per plant; RStY: Retted straw yield per plant; FY: Fibre yield per plant

capsules per plant, tillers per plant and 1000-seed weight over both checks, and days to 50% flowering, seed yield per plant, harvest index and oil content over standard check II), LC-2323 × LMH-62 (days to first flower, days to 50% flowering, days to 75% maturity and tillers per plant) and B-509 × Ariane (days to first browning of capsule, days to 75% maturity, biological yield per plant, retted straw weight per plant and fibre yield per plant over standard check I and primary branches per plant, seeds per capsule, biological yield per plant and fibre yield per plant over standard check II) (Tables 6 and 7).

Discussion

Heterosis signifies the percentage increase or decrease of F_1 over the better parent or the standard check, thereby identifying the best crosses, but fails to indicate the possible causes of the superiority of the hybrids. The genetic basis of the superiority of the best crosses is assessed on the basis of combining ability

effects. Thus, the comparison of the top-ranking hybrid combinations based on *per se* performance, SCA effects and heterosis revealed that, in general, the top-ranking hybrids based on *per se* (mean) performance also figured among the best combinations on the basis of standard heterosis (I and II). However, their superiority in respect of SCA was somewhat altered. Similar results were also observed by Pathania (1999). The preponderance of additive and additive × additive types of gene action might be a possible reason for such results. The crosses LC-2323 × LCK-9826 and B-509 × KL-221 performed best for both seed and fibre yield and their component traits, while LC-2323 × KL-210 was promising for seed yield and B-509 × Ariane for fibre yield, showing their superiority over seed type (I) and dual-purpose (II) checks, respectively (Table 8). A close relationship between *per se* performance and SCA effects and/or heterosis for yield and yield attributes was also reported by Rao and Singh (1983), Mishra and Rai (1993), Saraswat et al. (1993), Verma and Mahto (1996), Mahto and Rahman (1998), Pathania (1999), Kumar et al. (2000) and Sood (2004).

It can be concluded from the results that sufficient genetic variability was present in the lines under investigation. The non-significance of mean squares for line × environment, tester × environment and line × tester × environment for the majority of the traits suggested that the GCA effects of the parents and the SCA effects of the hybrids were not influenced by the environments. Combining ability studies revealed that the lines KL-221 and LCK-9826 were good general combiners for seed yield and most of its components, whereas B-509 and Ariane were good general combiners for fibre yield and most of its components. Among the specific cross combinations, B-509 × Flak-1 was outstanding for seed yield per plant and B-509 × KL-187 and LC-2323 × LCK-9826 for fibre yield per plant, with high SCA effects. In general, the hybrids excelled their respective parents and standard checks for most of the characters studied. Based on the comparison of mean performance, SCA effects and extent of heterosis (standard heterosis I and II), the hybrids LC-2323 × LCK-9826 and B-509 × KL-221 appeared to be the most promising for both seed and fibre yield. Other promising combinations were LC-2323 × KL-210 and B-509 × Ariane for seed and fibre yield, respectively. The superiority of LC-2323, LCK-9826, KL-221, B-509 and Ariane as good general combiners was further confirmed by the involvement of these parents in the desirable cross combinations. The promising combinations LC-2323 × LCK-9826 and B-509 × KL-221, identified on the basis of *per se* performance and superiority over standard checks for both seed and fibre yield, could be exploited to develop dual-purpose genotypes in linseed.

Table 8

Exploitable cross combinations based on *per se* performance, specific combining ability effects and standard heterosis for different traits in two environments

Traits	*Per se* performance		SCA effects		Standard heterosis I	Standard heterosis II
Days to first flower	LC-2323 × LMH-62 LC-2323 × KL-221 LC-2323 × LCK-9826 LC-2323 × Flak-1 LC-2323 × KL-187		B-509 × Ariane B-509 × KL-233		LC-2323 × LMH-62	B-509 × LMH-62 B-509 × Flak-1 B-509 × KL-221 B-509 × KL-187 B-509 × LCK-9826
Height at first flowering	Seed flax	Fibre flax	Seed flax	Fibre flax	–	–
	LC-2323 × KL-221 LC-2323 × LMH-62 LC-2323 × KL-178 LC-2323 × Flak-1 LC-2323 × KL-187	B-509 × Belinka B-509 × Ariane B-509 × Aoyagi LC-2323 × Ariane B-509 × Flak-1	B-509 × LCK-9826	LC-2323 × LCK-9826		
Days to 50% flowering	LC-2323 × LMH-62 LC-2323 × LCK-9826 LC-2323 × KL-221 LC-2323 × Flak-1 LC-2323 × KL-178		B-509 × Belinka B-509 × KL-233 LC-2323 × LCK-9826		LC-2323 × LMH-62 LC-2323 × LCK-9826 LC-2323 × KL-178	LC-2323 × LMH-62 LC-2323 × LCK-9826 LC-2323 × KL-221 LC-2323 × KL-178 LC-2323 × KL-187
Days to 75% maturity	LC-2323 × Belinka LC-2323 × LMH-62 B-509 × KL-221 B-509 × Belinka B-509 × LCK-9826		LC-2323 × LMH-62		LC-2323 × Belinka LC-2323 × LMH-62 B-509 × KL-221 B-509 × Belinka B-509 × LCK-9826	–
Plant height (cm)	Seed flax	Fibre flax	Seed flax	Fibre flax	–	–
	LC-2323 × LMH-62 LC-2323 × KL-221 LC-2323 × KL-233 LC-2323 × KL-178 LC-2323 × Janaki	B-509 × Ariane B-509 × Aoyagi B-509 × Belinka B-509 × Flak-1 B-509 × KL-187	B-509 × Ariane	LC-2323 × Ariane		
Technical height (cm)	Seed flax	Fibre flax	Seed flax	Fibre flax	–	–
	LC-2323 × LMH-62 LC-2323 × KL-221 LC-2323 × LCK-9826 LC-2323 × KL-233 LC-2323 × KL178	B-509 × Ariane B-509 × Belinka B-509 × Aoyagi B-509 × Flak-1 B-509 × Janaki	B-509 × Belinka LC-2323 × Flak-1	B-509 × Flak-1 LC-2323 × Belinka		

Table 8 continued

Primary branches per plant	LC-2323 × KL-221 B-509 × KL-221 LC-2323 × Ariane LC-2323 × LCK-9826 B-509 × LMH-62	–			LC-2323 × KL-221 LC-2323 × Ariane LC-2323 × LCK-9826	LC-2323 × KL-221 B-509 × KL-221 LC-2323 × Ariane LC-2323 × LCK-9826 B-509 × LMH-62
Capsules per plant	LC-2323 × KL-221 B-509 × Aoyagi B-509 × KL-221 LC-2323 × LCK-9826 B-509 × LMH-62	B-509 × LMH-62			LC-2323 × KL-221 B-509 × Aoyagi B-509 × KL-221 LC-2323 × LCK-9826 B-509 × LMH-62	LC-2323 × KL-221 B-509 × Aoyagi B-509 × KL-221 LC-2323 × LCK-9826 B-509 × LMH-62
Tillers per plant	LC-2323 × KL-221 LC-2323 × LMH-62 LC-2323 × LCK-9826 LC-2323 × Ariane LC-2323 × KL-178	–			LC-2323 × LMH-62 LC-2323 × KL-221 LC-2323 × LCK-9826 LC-2323 × KL-178	LC-2323 × LMH-62 LC-2323 × KL-221 LC-2323 × LCK-9826 LC-2323 × Ariane LC-2323 × KL-178
Seeds per capsule	B-509 × Aoyagi LC-2323 × LMH-62 B-509 × Ariane B-509 × Flak-1 B-509 × Janaki	B-509 × Flak-1 B-509 × Belinka B-509 × Aoyagi B-509 × Ariane LC-2323 × LMH-62			–	B-509 × Aoyagi LC-2323 × LMH-62 B-509 × Ariane B-509 × Flak-1 B-509 × Janaki
Seed yield per plant (g)	LC-2323 × LCK-9826 LC-2323 × KL-221 B-509 × KL-221 LC-2323 × KL-210 LC-2323 × Aoyagi	B-509 × Flak-1			LC-2323 × LCK-9826 LC-2323 × KL-221 LC-2323 × KL-210	LC-2323 × LCK-9826 LC-2323 × KL-221 B-509 × KL-221 LC-2323 × KL-210 LC-2323 × Aoyagi
1000-seed weight (g)	LC-2323 × LCK-9826 LC-2323 × KL-178 LC-2323 × KL-187 LC-2323 × Janaki LC-2323 × KL-221	–			LC-2323 × LCK-9826 LC-2323 × KL-178 LC-2323 × KL-187 LC-2323 × Janaki LC-2323 × KL-221	LC-2323 × LCK-9826 LC-2323 × KL-178 LC-2323 × KL-187 LC-2323 × Janaki LC-2323 × KL-221
Straw yield per plant (g)	Seed flax LC-2323 × LMH-62 LC-2323 × KL-187 LC-2323 × Flak-1 LC-2323 × Janaki LC-2323 × KL-233	Fibre flax B-509 × Aoyagi B-509 × LMH-62 LC-2323 × Ariane B-509 × Ariane B-509 × Flak-1	Seed flax LC-2323 × LMH-62 B-509 × Ariane	Fibre flax B-509 × LMH-62 LC-2323 × Ariane	–	–

Table 8 continued

Biological yield per plant (g)	B-509 × KL-221 B-509 × LMH-62 B-509 × Aoyagi B-509 × Flak-1 LC-2323 × Ariane	B-509 × LMH-62 B-509 × Flak-1 LC-2323 × Belinka	B-509 × KL-221 B-509 × LMH-62 B-509 × Aoyagi B-509 × Flak-1 LC-2323 × Ariane	B-509 × KL-221 B-509 × LMH-62 B-509 × Aoyagi B-509 × Flak-1 B-509 × KL-187
Harvest index (%)	LC-2323 × KL-210 LC-2323 × KL-187 LC-2323 × KL-233 LC-2323 × KL-221 LC-2323 × LMH-62	B-509 × Janaki LC-2323 × LMH-62	–	LC-2323 × KL-210 LC-2323 × KL-187 LC-2323 × KL-233 LC-2323 × KL-221 LC-2323 × LMH-62
Retted straw weight per plant (g)	B-509 × Aoyagi B-509 × LMH-62 B-509 × Flak-1 B-509 × Ariane LC-2323 × Ariane	B-509 × LMH-62 LC-2323 × Belinka LC-2323 × Ariane	–	B-509 × Aoyagi
Fibre yield per plant (g)	B-509 × KL-221 B-509 × Ariane B-509 × LMH-62 B-509 × KL-187 LC-2323 × LCK-9826	B-509 × KL-187 LC-2323 × LCK-9826	B-509 × KL-221 B-509 × Ariane B-509 × LMH-62 B-509 × KL-187 LC-2323 × LCK-9826	B-509 × KL-221 B-509 × Ariane
Oil content (%)	LC-2323 × LCK-9826 LC-2323 × Janaki LC-2323 × KL-221 LC-2323 × KL-233 LC-2323 × KL-187	LC-2323 × LCK-9826	–	LC-2323 × LCK-9826 LC-2323 × Janaki LC-2323 × KL-221 LC-2323 × KL-233 LC-2323 × KL-187

References

Allard, R. W. (1960): *Principles of Plant Breeding*. John Wiley and Sons, Inc., New York.

Anonymous (2004): *Annual Progress Report*. All India Co-ordinated Research Project on Oilseeds (Linseed) (ICAR), Project Coordinating Unit (Linseed), CSAUAT, Kanpur, 1 (Executive summary).

Anonymous (2008): *Production Year Book*. Food and Agricultural Organization, Rome http://faostat.fao.org/site

Badwal, S. S., Gupta, V. P. (1970): Gene effects governing inheritance of yield and its components in linseed (*Linum usitatissimum* L.). *Plant Sci.*, **2**, 36–51.

Bhateria, S., Pathania, A., Sharma, J. K., Badiyala, D., Bhandari, J. C. (2001): Combining ability for seed yield and its components in linseed (*Linum usitatissimum* L.). *J. Oilseeds Research*, **18**, 44–47.

Gill, K. S. (1987a): *Chemistry of Linseed*. Linseed Publication and Information Division, ICAR, New Delhi, pp. 301–316.

Gill, K. S. (1987b): Recent concepts in breeding self-pollinated crops. pp. 83–129. In: Gill, K. S., Khera, A. S., Verma, M. M. (eds.), *Proceedings of the First Symposium on Crop Improvement*, held at Punjab Agricultural University, Ludhiana on 23–27 February, 1987.

Hallauer, A. R., Miranda, J. B. (1981): *Quantitative Genetics in Maize Breeding*. Iowa State University Press, Ames, Iowa.

Jinks, J. L. (1983): Biometrical genetics of heterosis. pp. 1–46. In: Frankel, R. (ed.) *Heterosis*. Monographs on Theoretical and Applied Genetics, Springer Verlag, Berlin.

Kempthorne, O. (1957): *An Introduction to Genetical Statistics*. John Wiley and Sons, New York. 458 p.

Kumar, M., Singh, P. K., Singh, N. P. (2000): Line × tester analysis for seed yield and its components in linseed (*Linum usitatissimum* L.). *Annals of Agricultural Research*, **21**, 485–489.

Mahto, C., Rahman, M. H. (1998): Line × tester analysis of seed yield and its components in linseed (*Linum usitatissimum* L.). *J. Oilseeds Research*, **15**, 242–246.

Mather, K. (1973): *Genetical Structure of Populations*. Chapman and Hall, London.

Mishra, V. K., Rai, M. (1993): Estimates of heterosis for seed yield, components of seed and oil in linseed (*Linum usitatissimum* L.). *Indian J. Genet. and Plant Breeding*, **53**, 161–164.

Panse, V. G., Sukhatme, P. V. (1984): *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi. 381 p.

Pathania, A. (1999): *Genetic analysis involving exotic and indigenous linseed* (Linum usitatissimum *L.*) *genotypes*. MSc Thesis. CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H. P.).

Pillai, B., Khorgade, P. W., Narkhede, M. N. (1995): Genetic behaviour of yield and its components in linseed. *J. Oilseeds Res.*, **12**, 5–9.

Rai, M., Singh, H., Hegde, D. M. (2002): *Oilseeds and Oils – Research and Development Needs*. Indian Society of Oilseeds Research, Directorate of Oilseeds Research, Rajendranagar, Hyderabad, India.

Rao, S. K., Singh, S. P. (1983): Heterosis and inbreeding depression in linseed. *Indian J. Agric. Sci.*, **53**, 409–417.

Saraswat, A. V., Kumar, S., Verma, M. M., Virk, D. S., Chahal, G. S., (1993): Heterosis and inbreeding depression in some early hybrids of linseed. In: *Heterosis Breeding in Crop Plants. Theory and Application: Short Communications*. Symposium, Ludhiana, 23–24 February 1993, pp. 52–53.

Singh, P., Srivastava, A. N. (1987): Genetics of grain yield and oil content in linseed. *J. Oilseeds Res.*, **4**, 111–115.

Sood, S. P. (2004): *Genetic analysis of yield and its components in linseed* (Linum usitatissimum L.). MSc Thesis. CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H. P.).

Tandon, J. P. (1980): Problems and prospects of utilizing winter wheat in the Indian Wheat Programmes. *19th All India Wheat Research Workers Workshop, Surat.*

Thakur, H. L., Bhateria, S. (1991): Line × tester analysis for combining ability in linseed. J. Oilseeds Res., **8**, 14–19.

Thakur, H. L., Rana, N. D. (1987): Combining ability in linseed. *Indian J. Agric. Sci.*, **57**, 303–308.

Verma, A. K., Mahto, J. L. (1996): Hybrid vigour in linseed (*Linum usitatissimum* L.) for yield and yield attributes under rainfed and irrigated environments. *J. Tropical Agric.*, **34**, 54–57.

Yadav, R. K., Srivastava, S. B. L. (2002): Combining ability analysis over environments in linseed (*Linum usitatissimum* L.). *Crop Research*, **23**, 277–282.

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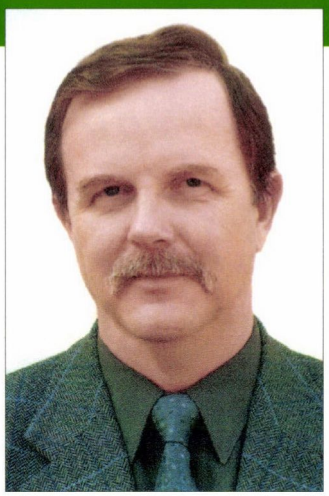

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CONTENTS

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OVEREXPRESSION OF THE ACYL-LIPID Δ12-DESATURASE GENE PROTECTS POTATO PLANTS FROM LOW TEMPERATURE DAMAGE

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The responses of plant leaves to chilling were studied in potato (*Solanum tuberosum* L., cv. Desnitsa) and in its transformants with the native *desA* gene that encodes the acyl-lipid Δ12-desaturase from the cyanobacterium *Synechocystis* sp. PCC 6803 and with the hybrid *desA* gene fused to the reporter gene of thermostable lichenase (*licB*M3) from *Clostridium thermocellum*. Cold stress caused a rapid and significant increase in superoxide production and lipid peroxidation (the content of conjugated dienes and malonic dialdehyde) in wild-type plants. By contrast no significant increase was detected in transformed plants under cold stress conditions. This can be attributed to the fact that the overexpression of the acyl-lipid Δ12-desaturase in transformed potato plants promotes fatty acid polyunsaturation and presumably averts the accelerated generation of the superoxide anion, thus suppressing lipid peroxidation under low-temperature stress

Abbreviations: CD, conjugated dienes, FA, fatty acid, MDA, malonic dialdehyde, NBT, Nitroblue tetrazolium, ROS, reactive oxygen species, SOD, superoxide dismutase, TBA, thiobarbituric acid, UFAs, unsaturated fatty acids.

Key words: Δ12-acyl-lipid desaturase, oxidative stress, lipid peroxidation, superoxide dismutase, transformed plants, thermostable lichenase

Introduction

The non-specific response of plants to low temperature is known to be associated with the activation of intracellular oxidative processes involving reactive oxygen species (ROS) and is named oxidative stress (Scandalios, 1993; Blokhina et al., 2003). The accumulation of ROS provides a basis for the peroxidation of the unsaturated fatty acids (UFAs) of membrane phospholipids. A decrease in the amount of UFAs during lipid peroxidation elevates membrane viscosity and pro-

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motes lipid transition from a liquid crystalline phase to a gel phase (Los and Murata, 1998). The latter may increase proton permeability, diminish membrane electric conductance, and ultimately cause the inactivation of membrane enzymes. During the long-term action of stress factors, these changes become irreversible and may lead to multiple disturbances in biological systems and eventual plant death (Suzuki and Mittler, 2006).

The low temperature acclimation of plant cells is mainly based on their ability to increase the amount of UFAs in membrane lipids. This adaptive mechanism is thought to compensate for the low temperature-dependent loss of membrane integrity by decreasing the lateral packing order of acyl chains within the membrane interior (Hazel, 1997). Desaturases exhibit high specificity to the carbon chain length and to the location of the double bond. The fatty acid (FA) chains of cell membrane lipids usually contain 16 or 18 carbon atoms. The first double bond is always formed at the Δ9 position, while the second bond appears at position Δ12; subsequent double bonds arise at positions Δ15, ω3 or Δ6. In the cyanobacterium *Synechocystis* sp. PCC6803, double-bond formation is due to the activity of acyl-lipid desaturases, such as Δ9-desaturase (*desC* gene), Δ12-desaturase *(desA),* Δ15-desaturase *(desB)* and Δ6-desaturase *(desD)*. Plants, animals and fungi contain several types of desaturases that produce double bonds in other positions of the FA chains (Murata and Wada, 1995; Los and Murata, 1998). Δ12-desaturase is known to be a key enzyme for the biosynthesis of dienoic FAs (Wada et al., 1990). The presence of linoleic acid (C18:2) and, consequently, the activity of Δ12-desaturase were found to be crucial factors for the formation of the organism-specific membrane structure (Nishida and Murata, 1996).

It was previously demonstrated that the overexpression of the *desA* gene from *Synechocystis* for the acyl-lipid Δ12-desaturase in potato changed the FA composition and elevated the content of unsaturated FAs in the leaves of various transgenic lines. In particular, the content of linoleic ($18:2^{\Delta 9,12}$) acid rose by 35% (line DesA-LicBM3, transformed with desaturase fused to lichenase) and by 40% (line DesA, transformed with desaturase alone); an increased level of linolenic ($18:3^{\Delta 9,12,15}$) acid was observed in line DesA-LicBM3 and line DesA, the increment being 41 and 12%, respectively. The total level of unsaturated FAs in line DesA-LicBM3 and line DesA exceeded their level in wild-type plants by 42 and 24%, respectively (Maali-Amiri et al., 2007).

It should be noted that some gene products do not display any enzymatic activity when cloned into the cells of heterologous hosts, or it could only be determined using specific approaches. To some extent, this is also true of the *des*A gene. Therefore, a novel approach was suggested to construct experimental models to create transgenic plants tolerant to stresses. This approach is based on the construction of hybrid genes comprising the gene of interest, the *des*A gene, which is translationally fused with the reporter gene for thermostable lichenase (*lic*BM3). This reporter system displays several advantages: it permits the use of simpler and more sensitive methods for the analysis of hybrid gene expression, thus accelerating the selection of transgenic organisms and the estimation of the

expression level of hybrid genes and the molecular weights of their protein products (Goldenkova et al., 2003).

The aims of this study were: (1) to assess the protective effect of polyunsaturated FAs in the cell membranes under oxidative stress induced by low temperature in potato plants overexpressing the *desA* gene from *Synechocystis* sp. PCC 6803, and (2) to estimate the rates of superoxide anion generation, the activity of superoxide dismutase (SOD) and lipid peroxidation at low temperature.

Materials and methods

Plant material

Investigations were conducted with transformed potato plants (*Solanum tuberosum* L.) cv. Desnitsa harbouring a hybrid *desA-licB*M3 gene (abbreviated as the line DesA-LicBM3) and line DesA comprising a native cyanobacterial gene of acyl-lipid Δ12-desaturase from *Synechocystis* sp. PCC 6803 (abbreviated as the line DesA). In line DesA-LicBM3, the desaturase sequence was translationally fused with the sequence of the reporter gene *licB*M3 of thermostable β-1,3-1,4-glucanase (lichenase) from *Clostridium thermocellum*. The transformed plants also harboured the kanamycin resistance gene *nptII*. The genes were under the control of a strong constitutive 35S CaMV promoter. Non-transformed plants (called wild-type plants) of the same cultivar were used as control samples.

Construction of plant expression vectors

Standard protocols were used for molecular cloning and PCR (Maniatis et al., 1982). Restriction enzymes, T4 DNA ligase and *Pfu* DNA polymerase were applied according to the manufacturer's protocols (Promega, USA; Fermentas, Lithuania). The nucleotide sequence of the *licB*M3 reporter gene was amplified by PCR, using the pQE30-*lic*BM2-KM2-Mys25 plasmid produced earlier (Goldenkova et al., 2003) as a template and the *licB*M3-sense-*Bam*HI (GGATCCGT GGTAAATACGCCTTTTG) and *licB*M3-anti-*Sph*I (GCATGCGTTAGGATAGTATTTTACATA TTCG) primers. The amplified fragment was cloned into the pGEM-T plasmid (Promega), thus producing the pGEM-*licB*M3 plasmid. The sequence of the native *des*A gene was obtained by PCR, using genomic DNA from *Synechocystis* sp. PCC 6803 (Wada et al., 1990) as a template and the *desA*-sense-*Xho*I (CTCGAGATGACTGCCACGATTCC) and *desA*-anti-*Bgl*II (AGATCTTTGAA CTTTTTTCAGGGAGCC) primers. Synthetic oligonucleotides were synthesized by Evrogen, Rus-

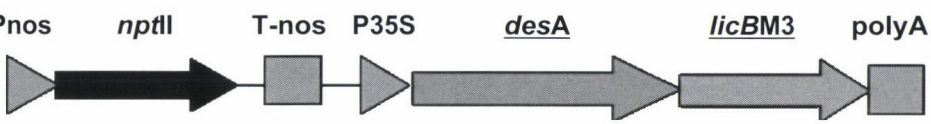

Fig. 1. Scheme of plant expression vectors for transformation of plants harbouring the hybrid *desA-licB*M3 gene

Pnos – promoter of the *npt*II gene; *npt*II – the gene encoding neomycin transferase II; Tnos – terminator of the *npt*II gene; P35S – constitutive 35S CaMV promoter for expression of the gene of interest; *desA* – the gene encoding D12 acyl-lipid desaturase; *licB*M3 – sequence of the reporter gene encoding thermostable β-1,3-1,4-glucanase (lichenase) from *Clostridium thermocellum;* polyA – site for polyadenylation

sia. The amplified fragment of the *desA* gene was cloned into the pGEM-T vector, forming the pGEM*desA* plasmid. Thereafter, the *Xho*-I-*Sph* I fragment of this plasmid was cloned into the pIK vector, preliminarily cleft by *Xho*I and *Sph*I endonucleases, forming the pIK-*desA* vector (Goldenkova et al., 2003). The pIK-*desA* vector was digested by *Xho*I and *Xba*I, and the *Xho*I-*Xba*I fragment comprising the sequence of the *desA* gene was cloned into the pBISN1-IN vector for plant transformation, which was preliminarily digested by the *Xho*I and *Xba*I endonucleases. As a result, the expression vector pBISN1-IN-*desA* was obtained, where the gene encoding D12 desaturase was under the control of a strong constitutive 35S CaMV promoter (Fig. 1).

The construction of the pBISN1-IN*desA-licB*M3 expression vector was performed in several steps. First, the *Bam*HI-*Sph*I fragment of the pGEM-*licB*M3 plasmid was cloned into the pGEM-*desA* plasmid preliminarily digested with *Bgl*II and *Sph*I to form the pGEM-*des*A-*licB*M3 plasmid. Thereafter, the *Xho*I-*Sph*I fragment of pGEM-*des*A-*licB*M3 was cloned into the pIK vector digested with *Xho*I and *Sph*I, thus forming the pIK-*desA-licB*M3 vector. The *Xho*I-*Xba*I fragment comprising the sequence of the hybrid gene *desA-licB*M3 was cloned into pBISN1-IN (BV Tech) at the same restriction sites, resulting in the construction of the pBISN1-IN-*desA-licB*M3 expression vector (Fig. 1). The resultant genetic constructants were transferred into *Agrobacterium tumefaciens* strain AGLO (Lazo et al., 1991). The *nptII* gene was used as a selective one. Sequencing confirmed the correctness of native and hybrid gene cloning.

Production of transformed plants

Microtubers of potato were produced *in vitro,* using the technology developed at the Department of Cell Biology and Biotechnology of the Institute of Plant Physiology RAS (Deryabin et al., 1997). Segments of these microtubers (explants) were used for transformation, according to a modified protocol described earlier (Van Lijsebettens and Valvekense, 1987). The explants were placed in Petri dishes with liquid medium designed for callus formation, containing 5 mg/l IAA and devoid of antibiotics. Night culture (2 ml) of *Agrobacterium* strain AGLO (Lazo et al., 1991) was added, and incubation was performed for 15 min with constant stirring. Then the explants were transferred to solid medium with the same composition and incubated at 18–20°C for 48 h under dim light. To induce morphogenesis, the explants were transferred to agar-solidified medium containing zeatin (1 mg/l), cefotaxime (700 mg/l) to suppress bacterium growth, and kanamycin (10 mg/l) as a selective agent. The Petri dishes were incubated at 22°C under 100 mol quanta/m^2·sec illumination. Subculturing on morphogenic medium was performed every two weeks. After 2–3 cycles of subculturing in the presence of 15 mg/l kanamycin, shoots were obtained, which were transferred to MS medium (Murashige and Skoog, 1962) containing 50 mg/l kanamycin. Rooted plants with normal green leaves were used for further molecular and biological analyses. The selection of primary transformants, which expressed the *desA* and *desA-licB*M3 genes, was performed using PCR on genomic plant DNA and the assay of reporter protein activity (Goldenkova et al., 2003; Sotchenkov et al., 2005). As a result, the T1 plant generation was obtained for line DesA-LicBM3, harbouring a hybrid *desA-licB*M3 gene, and for line DesA, with a native *desA* gene.

Plant growth and treatments

The plants were grown *in vitro* in a growth chamber (luminescent lamps producing white light, 100 mol quanta/m^2·sec illumination, 16/8-h day/night regime, 22°C, 60% relative humidity) for 5 weeks in MS medium containing 0.7% agar, 2% sucrose, 0.5 mg/l thiamine-HCl, 0.5 mg/l pyridoxine and 60 mg/l myo-inositol.

During the cold treatment, the plants were placed in a climatic chamber (MIR-153, Sanyo, Japan) preliminarily chilled to 0°C. During further treatment, the temperature was lowered gradually to –9°C (at the rate of 0.3°C/min), and the plants were incubated at this temperature for 15 min.

The cooling regime (i.e. the combination of temperature and incubation period) was chosen in a set of preliminary experiments. Since potato is a cold-tolerant species, the long-term action of low temperature suppresses plant growth without having a detrimental influence on the plants. Freezing temperatures that induce ice formation result in plant death. Therefore, the chilling treatment (–9°C for 15 min) should involve supercooling which induces discernible changes in cell membranes in cold-tolerant plants. The cooling regime adopted in the present experiments made it possible to differentiate the genotypes examined in terms of their resistance to low-temperature stress. It should be noted that the plants survived the chilling treatment, and no ice formation was observed during the experiments.

PCR analysis of transgenic potato plants

DNA was extracted and purified from the leaf material as described in the literature (Querci et al., 2006). The DNA concentration was measured by UV absorption at 260 nm and the purity was evaluated by the A_{260nm}/A_{280nm} ratio using a BioPhotometer (Eppendorf, Germany). Multiplex PCR assays with target DNA isolated from transgenic plants were carried out as described in a previous work (Berdichevetz et al., 2010). The leaves of non-transformed plants served as negative controls.

Lichenase activity assay

Plant protein extracts were obtained with 50 or 100 mM Tris-HCl, pH 8.0 (Piruzian et al., 2002). The protein concentration in bacterial and plant extracts was measured with the Bradford method (Bradford, 1976) with BioRad Dye (BioRad, USA), and bovine serum albumin (BSA) (Sigma, USA) was used to construct the calibration curve.

The lichenase activity in the extracts was determined at 65°C using lichenan as substrate as described by Piruzian et al. (2002). The reducing sugars released from the substrate were assayed with the reagent dinitrosalicyl (DNS). The reaction mixture, containing 200 µl of 0.5% lichenan and 100 µl of the protein sample, was incubated for 10–20 min. Then 1.2 ml of the DNS reagent was added, and the mixture was heated at 100°C for 15 min. The concentration of the coloured product was determined with a spectrophotometer.

Lichenase activity was measured by the plate test method described by Wood and Bhat (1988) with modifications. Plant extracts (20 µl) were placed into wells on Petri plates containing agar with the substrate lichenan. The plates were then incubated for 1–5 h at 65°C, stained for 15 min with 0.5% Congo Red (Sigma, USA) solution and washed with 1 M NaCl until transparent spots of the hydrolysed substrate became apparent.

Protein electrophoresis

The protein extracts from transgenic plants were characterized by the enzymogram method as described earlier (Piruzian et al., 2002). Protein electrophoresis was performed under denaturing conditions (Laemmli, 1970). Zymograms were obtained by separating the protein in 10% SDS-PAGE containing 0.1% lichenan, followed by enzyme staining (Piruzian et al., 2002).

Determination of reactive oxygen species

The rate of ROS (mostly superoxide anion) generation was determined by a method based on the adrenaline colour reaction during the oxidative conversion of adrenaline to adrenochrome

(Bligh and Dyer, 1959). The rate of superoxide anion production was expressed in relative units (1 rel. unit = 10^{-3} absorbance unit per min). The specificity of the reaction was checked by the addition of superoxide dismutase (SOD, 100 enzyme activity units).

Assay of SOD activity

SOD activity was determined as described by Kumar and Knowles (1993) with some modifications. The method is based on the generation of superoxide anion radicals in the reaction of riboflavin photooxidation stimulated by nitroblue tetrazolium (NBT), which serves as an indicating scavenger. During this reaction NBT is reduced to formazan, which turns blue-violet. If the system contains SOD, the enzyme catalyses the dismutation of the superoxide anion radical known to hamper formazan formation. The highest formazan production is observed if the system contains no SOD. The SOD activity was estimated from the extent of inhibition of NBT reduction at $\lambda = 560$ nm (absorption peak of formazan). The values were calculated from the expression lg(D1/D2)/lg2V (where D1 is the absorbance of the control mixture without plant extract in optical density units, D2 is the absorbance of a test sample and V is the sample volume in millilitres) and expressed in activity units per gram fresh leaf weight.

Content of lipid peroxidation products

The content of conjugated dienes (CD), the primary lipid peroxidation products, was determined by a method based on the ability of double and triple bonds in lipid hydroperoxides to absorb ultraviolet light with characteristic absorption peaks at 232 and 275 nm. The chloroform extracts were obtained by the modified method of Bligh and Dyer (1959). The concentrations of CD were calculated according to the Lambert–Beer law: $D=C\cdot\varepsilon\cdot l$, $C=D/\varepsilon\cdot l$, where C is the concentration of CD, D is the optical density (absorbance), ε is the molar extinction coefficient ($2.21\cdot10^5$ $cm^{-1}M^{-1}$), l is an optical path length (1 cm), and A is the extent of dilution. The content of CD was expressed in mmol per gram of fresh weight of leaves (Kates, 1972).

The level of malonic dialdehyde (MDA) was determined by means of the colour reaction with thiobarbituric acid (TBA) (Heath and Packer, 1968). MDA is a dominant stable product of lipid peroxidation and the main component of TBA-reactive products. The optical density of the samples was measured at a wavelength of 532 nm. The content of MDA was expressed in μmol/g fr wt, with the coefficient of molar extinction being equal to $1.56\cdot10^5$ $cm^{-1}M^{-1}$. Leaf samples were taken from the middle part of 3–5 plants.

Statistical analysis

The results were treated statistically using Student's t-test for paired samples, $P = 0.05$. The values were the means of typical experiments comprising ten replicates and their standard errors. Differences reliable at a 95% level of significance are discussed.

Results and discussion

The primary plant transformants were screened using the multiplex PCR (MPCR) assay with the target DNA, which was isolated from transgenic potato plants. The results showed specific amplicons of the expected size for *desA* (949

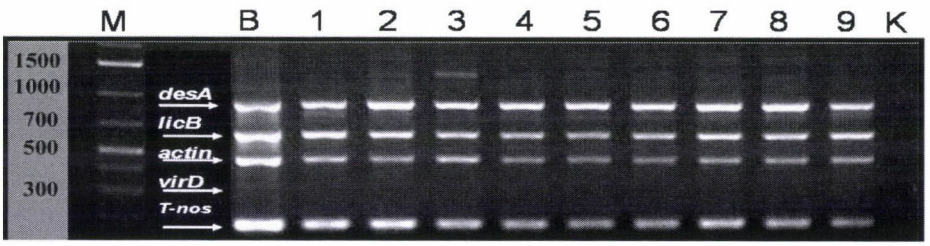

Fig. 2. Multiplex PCR (MPCR) on the genomic DNA of potato transformants (numerals denote independent transformants)
desA – target gene of Δ12 acyl-lipid desaturase; *licB* – reporter gene encoding thermostable β-1,3-1,4-glucanase (lichenase) from *Clostridium thermocellum; actin* – housekeeping gene; *T-nos* – terminator of the *npt*II gene; *virD* – virulence gene from the *Agrobacterium tumefaciens* chromosome; *M* – molecular weight marker; *B* – plant expression vector; *K* – control plants

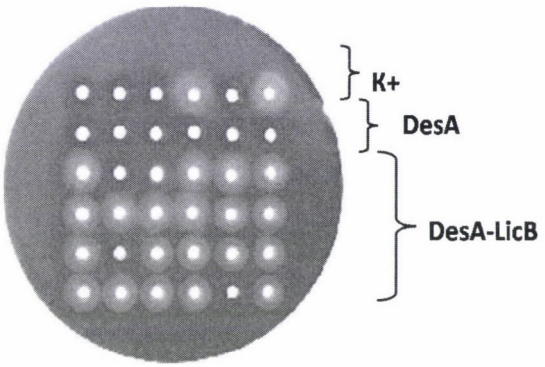

Fig. 3. Plate test for lichenase activity in leaf extracts of the different lines of transformants
K+ – positive control (extract from transformants of potato plants expressing the reporter gene *lic*BM3); DesA – lines of transformants with the native *des*A gene expression; DesA-LicB – lines of transformants with the fusion gene (*des*A-*lic*BM3) expression

bp), *licB*M3 (642 bp) and T-nos (188 bp) (Fig. 2). The MPCR method for the detection of *desA-licB*M3 genes makes it possible to detect *Agrobacterium* contamination and to confirm the quality of plant genomic DNA. MPCR demonstrated specific amplicons of the expected size for the housekeeping gene (391 bp), but did not detect specific amplicons for the *virD* gene of *Agrobacterium tumefaciens* (291 bp), indicating the integration of the target gene into the genome of transgenic potato plants.

The production of fused proteins in transgenic potato plants was shown using the plate test and the enzymogram technique (Figs. 3 and 4).

Lichenase activity (transparent spots around the holes) could be seen to be present in protein extracts of the leaf tissue of DesA-LicBM3 transformants (with hybrid *desA-licBM3* gene expression) and LicB transformants (with reporter

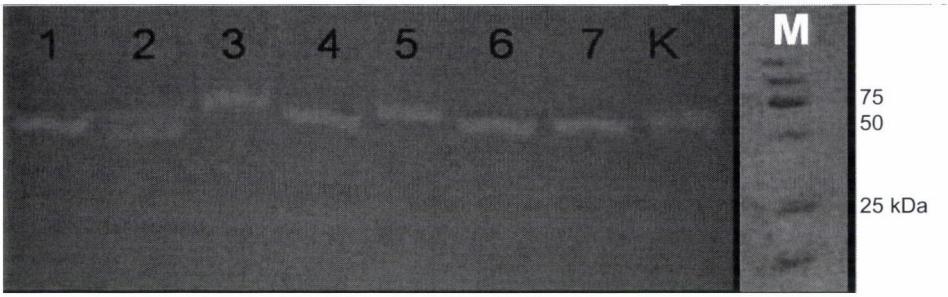

Fig. 4. Enzymogram of protein lysates derived from the cells of potato transformants (DesA-LicB line)
1–7 – samples of protein lysates of independent plant transformants; K – positive control (extract from bacterial transformant *pET32-des*A-*licB*M3); M – molecular weight marker

*licB*M3 gene expression only) (Fig. 3). The activity of lichenase as part of a fused DesA-LicBM3 protein, which was identified using the plate test method, allowed the selection of plants that express the hybrid *desA-licB*M3 gene. It should also be noted that lichenase activity was not revealed in all the primary transformants originally analysed with MPCR.

To prove that the potato transformants efficiently synthesize the full-length fusion protein DesA-LicBM3, the protein extracts isolated from transformant plants were analysed with the enzymogram method (Fig. 4).

It was confirmed that the fusion protein DesA-LicBM3 was synthesized in the DesA-LicBM3 plant transformant cells. Its molecular weight was approximately 63 kDa, which corresponds to the theoretically calculated molecular mass for this fusion protein.

As a result, several independent primary transformants of potato that expressed *desA* and *desA-licB*M3 genes were selected. The lines of these transformants were designated as DesA and DesA-LicBM3, respectively.

The structural and functional conditions of the cell membranes under low-temperature stress determine the viability of the plants (Nishiyama et al., 2006). However, low temperature may induce oxidative stress, which leads to uncontrolled ROS-mediated free-radical processes. The superoxide anion is one of the first substances generated by free-radical processes. The exposure of cells to cold conditions leads to the uncoupling of various pathways related to electron transport (Suzuki and Mittler, 2006). An extra electron, generated in the electron transport chain of mitochondria and chloroplasts (Foyer et al., 2002) and in the NADPH-oxidase pathways (Apel and Hirt, 2004), "overflows" to molecular oxygen, giving rise to superoxide. The superoxide thus generated is a basis for the production of other ROS (Pastori et al., 2000). The rates of ROS production (with the superoxide anion as the dominant ROS species) in the leaves of wild-type and transformed plants exhibited little variation under standard conditions (Fig. 5a). After short-term exposure to chilling temperature (–9°C for 15 min), the rate of superoxide production increased by 42% in wild-type plants, but the increase was

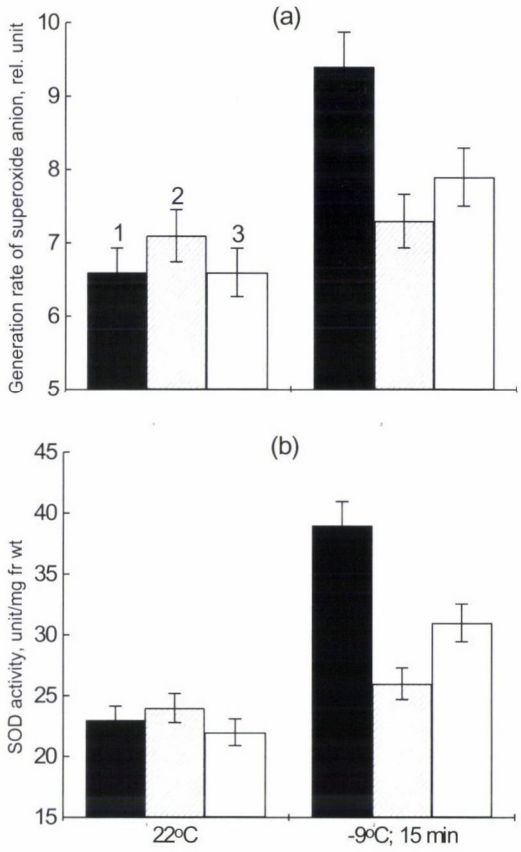

Fig. 5. Generation rates of superoxide anion (a) and activity of SOD (b) in potato leaves of wild-type plants (1) and plants transformed with the *desA* gene encoding acyl-lipid Δ12-desaturase from cyanobacterium *Synechocystis* sp. PCC 6803, line DesA (2) and line DesA-LicBM3 (3), at 22°C and after low-temperature stress (–9°C, 15 min)

insignificant in DesA-LicBM3 plants, and the rate remained virtually unchanged in DesA plants. This may provide evidence that the chilling-induced oxidative stress was significantly weaker in transgenic plants than in wild-type plants. In this respect, the rate of ROS production (superoxide anion in particular) is an important indicator of the initiation of low-temperature-induced oxidative stress. The lack of significant stimulation of superoxide anion production during low-temperature stress in potato plants transformed with the cyanobacterial gene for Δ12-desaturase (Fig. 5a) presents evidence for a lower level of oxidative stress in the transformants, compared to wild-type plants. Thus, potato plants that express the Δ12-desaturase gene differ from the wild-type plants in the reduced production of ROS during low-temperature stress. This demonstrates the important protective role of fatty acid unsaturation in the early stages of oxidative stress.

SOD activity was also measured in plants chilled to –9°C for 15 min (conditions that do not allow ice formation). Under normal growth conditions (22°C), the enzyme activities in the wild-type and transformed plants were fairly similar (Fig. 5b). However, during hypothermia, the SOD activity increased significantly in the leaves of wild-type plants compared to that in the leaves of DesA plants. This observation is consistent with the significant increase in ROS content upon the accelerated generation of radical oxygen species (Fig. 5a).

SOD is one of the principal enzymes in the antioxidant defence system, as it utilizes superoxide radicals (Suzuki and Mittler, 2006). SOD activity significantly increases in plants under various stress conditions. In the present experiments, such an increase in the enzyme activity was observed in wild-type plants during

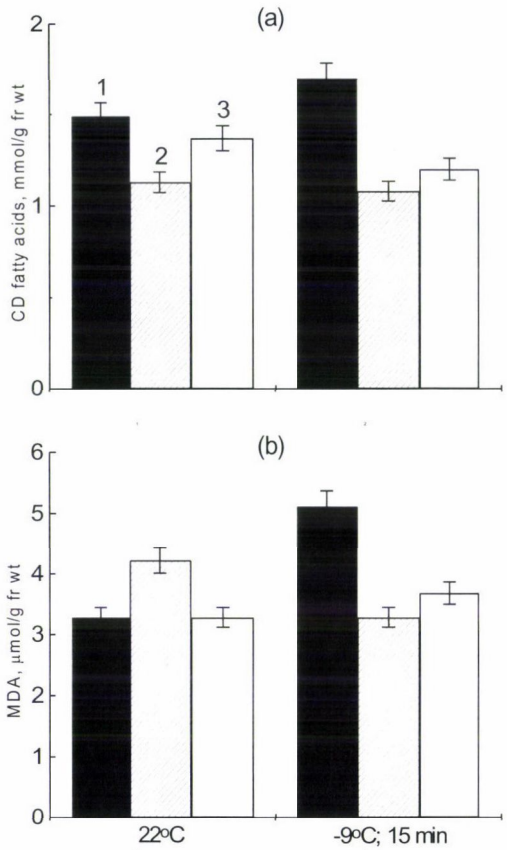

Fig. 6. Content of lipid peroxidation products in potato leaves of wild-type plants (1) and plants transformed with the *desA* gene encoding acyl-lipid Δ12-desaturase of cyanobacterium *Synechocystis* sp. PCC 6803, line DesA (2) and line DesA-LicBM3 (3), at 22°C and after low-temperature stress (–9°C, 15 min)
a) content of diene conjugates (CD); b) content of malonic dialdehyde

hypothermia, whereas the increase in leaf SOD activity was less obvious in DesA plants (Fig. 5b). It should be noted that the transcription of most SOD genes (including $Cu/ZnSOD_{cyt}$) is sensitive to chilling-induced oxidative stress (Tsang et al., 1991). It is also strongly induced by heat shock, as well as during the recovery period after cold stress. In both cases the response is essentially independent of light. According to Tsang et al. (1991), this implies that the cytosol may be the main place of ROS formation under temperature stress conditions. This could explain the great increase in cold-shock-inducible SOD activity displayed by wild-type plants compared to transformants. The production rate of superoxide (Fig. 5a) during –9°C treatment was higher in the wild-type plants than in the transformants. Figures 5a and 5b demonstrate a similar tendency in superoxide formation and SOD activity during the exposure of plants to low temperatures. Thus, cold treatment did not cause severe oxidative stress in transgenic plants, although this was evident in wild-type plants.

It was expected that the diminished generation of ROS during cold-induced oxidative stress in transformed plants should result in the lower production of lipid peroxidation products in the transformants compared to the wild-type plants. Indeed, the content of CD under normal growth conditions (22°C) in the leaves of wild-type plants was higher than in leaves of the transformants (Fig. 6a). Moreover, during the oxidative stress induced by low temperature, the concentration of CD in leaves of wild-type plants continued to increase. In contrast, the transformed plants exhibited comparatively elevated resistance to low temperature: the content of CD in transformed plants subjected to chilling was much lower than in cold-treated wild-type plants, and it even decreased slightly below the level observed in the transformed plants under normal temperature conditions. It should be noted that diene conjugation is a process based on the formation of conjugated double bonds between fatty acid molecules. Since such bonds are formed during lipid peroxidation, which involves lipid hydroperoxide generation, the assay of diene conjugation can provide a measure of the rate of these processes. The determination of CD is important for the assessment of lipid peroxidation, as it characterizes the early stages of oxidation (Kates, 1972).

Apart from CD, the concentration of MDA, as a final product of lipid peroxidation, is a principal marker of plant resistance to the oxidative stress induced by low temperatures. The content of MDA in the leaves of wild-type and transformed plants did not differ significantly under normal conditions. However, under low-temperature stress (–9°C for 15 min), the MDA content increased markedly in wild-type plants, while it remain unchanged in DesA-LicBM3 and DesA plants (Fig. 6b). This finding leads to the conclusion that the heterologous Δ12-desaturase expressed in potato plants stabilizes the structure of cell membranes by increasing the polyunsaturation of FAs in membrane lipids and thus preventing electron overflow to oxygen and the associated superoxide production.

Conclusions

The expression of the heterologous gene for the acyl-lipid Δ12-desaturase of *Synechocystis* in potato plants leads to an increase in the proportion of unsaturated fatty acids in membrane lipids and prevents the generation of ROS species under cold stress. It protects the membranes against injuring agents, thereby suppressing lipid peroxidation and destruction, which should eventually improve plant resistance to low-temperature stress.

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References

Apel, K., Hirt, H. (2004): Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant. Biol.,* **55**, 373–399.

Berdichevetz, I. N., Shimshilashvili, C. R., Gerasymenko, I. M., Sindarovska, Y. R., Sheludko, Y. V., Goldenkova-Pavlova, I. V. (2010): Multiplex PCR assay for detection of recombinant genes encoding fatty acid desaturases fused with lichenase reporter protein in GM plants. *Analytical and Bioanalytical Chemistry,* **397**, 2289–2293.

Bligh, E., Dyer, W. I. (1959): Rapid methods of total lipid extraction and purification. *Can. J. Biochem. Physiol.,* **37**, 911–917.

Blokhina, O., Virolainen, E., Fagerstedt, K. V. (2003): Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.,* **91**, 179–194.

Bradford, M. M. (1976): A rapid sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* **72**, 248–254.

Deryabin, A. N., Oreshnikov, A. V., Yur'eva, N. O., Butenko, R. G. (1997): Potato stolone growth and microtuber inducting under different *in vitro* cultivating regimes. *Dokl. Akad. Nauk.,* **355**, 841–843.

Foyer, C. H., Vanacker, H., Gomez, L. D., Harbinson, J. (2002): Regulation of photosynthesis and antioxidant metabolism in maize leaves at optimal and chilling temperatures: Review. *Plant Physiol. Biochem.,* **40**, 659–668.

Goldenkova, I. V., Musiichuk, K. A., Piruzyan, E. S. (2003): Bifunctional reporter genes: construction and expression in pro- and eukaryote cell. *Mol. Biol.,* **37**, 1–9.

Hazel, J. R. (1997): Thermal adaptation in biological membranes: Beyond homeoviscous adaptation. pp. 57–101. In: Williams, J. S. (ed.), *Advances in Molecular and Cell Biology.* JAI Press, New York.

Heath, R. L., Packer, L. (1968): Photoperoxidation in isolated chloroplasts. 1. Kinetics and stoichoimetry of fatty acid peroxidation. *Arch. Biochem. Biophys.,* **125**, 189–198.

Kates, M. (1972): Techniques of lipidology: Isolation, analysis, and identification of lipids. pp. 355–356. In: Work, T. S., Work, E. (eds.), *Laboratory Techniques in Biochemistry and Molecular Biology.* North-Holland/American Elsevier, New York.

Kumar, G. N., Knowles, N. R. (1993): Changes in lipid peroxidation and lipolytic and free-radical scavenging enzyme during aging and sprouting of potato (*Solanum tuberosum* L.) seed-tubers. *Plant Physiol.,* **102**, 115–124.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**, 680–685.

Lazo, G. R., Stein, P. A., Ludwig, R. A. (1991): A DNA transformation-competent *Arabidopsis* genomic library in *Agrobacterium*. *Agrobacterium Biotechnol.,* **9**, 963–967.

Los, D. A., Murata, N. (1998): Structure and expression of fatty acid desaturases. *Biochim. Biophys. Acta,* **1394**, 3–15.

Maali-Amiri, R., Goldenkova-Pavlova, I. V., Yur'eva, N. O., Pchelkin, V. P., Tsydendambaev, V. D., Vereshchagin, A. G., Deryabin, A. N., Trunova, T. I., Los, D. A., Nosov, A. M. (2007): Lipid fatty acid composition of potato plants transformed with the 12-desaturase gene from cyanobacterium. *Rus. J. Plant. Physiol.,* **54**, 600–606.

Maniatis, T., Frisch, E. F., Sambrook, O. (1982): *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Lab., Cold Spring Harbor. 545 pp.

Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.,* **15,** 473–497.

Murata, N., Wada, H. (1995): Acyl-lipid desaturases and their importance in the tolerance and acclimatization to cold of cyanobacteria. *Biochem. J.,* **308**, 1–8.

Nishida, I., Murata, N. (1996): Chilling sensitivity in plants and cyanobacteria: The crucial contribution of membrane lipids. *Annu. Rev. Plant. Physiol. Plant. Mol. Biol.,* **47**, 541–568.

Nishiyama, Y., Allakhverdiev, S. I., Murata, N. (2006): A new paradigm for the action of reactive oxygen species in the photoinhibition of photosystem II. *Biochem. et Biophys. Acta,* **1757**, 742–749.

Pastori, G., Foyer, C. H., Mullineaux, P. (2000): Low-temperature induced changes in the distribution of H_2O_2 and antioxidants in the bundle sheath and mesophyll cells of maize leaves. *J. Exp. Bot.,* **51**, 107–113.

Piruzian, E. S., Goldenkova, I. V., Mysiychuk, K. A., Kobets, N. S., Arman, I. P., Bobrysheva, I. V., Chekhuta, I. F., Glazkova, D. (2002): A reporter system for procaryotic and eucaryotic cells based on the thermostable lichenase from *Clostridium thermocellum*. *Mol. Genet. Genomics,* **266**, 778–786.

Querci, M., Jermini, M., Van der Eede, G. (2006): *The Analysis of Food Samples for the Presence of Genetically Modified Organisms*. Office for Official Publications of the European Communities, Luxembourg. 229 pp.

Scandalios, J. G. (1993): Oxygen stress and superoxide dismutases. *Plant Physiol.,* **101**, 7–12.

Sotchenkov, D. V., Goldenkova, I. V., Mirakholi, N., Volkova, L. V. (2005): Modification of gene *SD2* sequence in sunflowers and its expression in bacterial and yeast cells. *Genetika,* **41**, 1453–1461.

Suzuki, N., Mittler, R. (2006): Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. *Physiol. Plant.,* **126**, 45–51.

Tsang, E. W. T., Bowler, C., Herouart, D., Camp, W. V., Villaroel, R., Genetello, C., Van Montagu, M., Inzé, D. (1991): Differential regulation of superoxide dismutases in plants exposed to environmental stress. *The Plant Cell,* **3**, 783–792.

Van Lijsebettens, M., Valvekense, D. (1987): Tobacco leaf disk infection with *Agrobacterium tumefaciens*. *EMBO Practical Course on Plant Molecular Biology,* Gent, Belgium. pp. 15–18.

Wada, H., Gombos, Z., Murata, N. (1990): Enhancement of chilling tolerance of a cyanobacterium by genetic manipulation of fatty acid desaturation. *Nature,* **347**, 200–203.

Wood, T. M., Bhat, K. M. (1988): Methods for measuring cellulase activities. *Methods Enzymol.,* **160**, 87–112.

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PHYTOREMEDIATION: ENHANCED CADMIUM (Cd) ACCUMULATION BY ORGANIC MANURING, EDTA AND MICROBIAL INOCULANTS (*Azotobacter* sp., *Pseudomonas* sp.) IN INDIAN MUSTARD (*Brassica juncea* L.)

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Phytoremediation is an approach designed to extract excessive heavy metals from contaminated soils through plant uptake. Cadmium (Cd) is among the elements most toxic to living organisms. Health hazards associated with the lethal intake of Cd include renal (kidney) damage, anaemia, hypertension and liver damage. A greenhouse experiment was carried out with Indian mustard (*Brassica juncea)* grown on artificially spiked soil (100 μg Cd g^{-1}) with EDTA (2 mmol kg^{-1} in 5 split doses), FYM, vermicompost (VC) and microbial inoculants (MI) such as *Azotobacter* sp. and *Pseudomonas* sp. The growth of *Brassica juncea* L. was better in soil amended with FYM or VC as compared to unamended Cd-polluted soil. Growth was slightly suppressed in EDTA-treated soil, whereas it was better after treatment with MI. The application of FYM and VC increased the dry matter yield of Indian mustard either alone or in combination with microbial inoculants, while that of EDTA caused a significant decrease in the biomass of Indian mustard. The application of microbial inoculants increased the dry matter yield of both the roots and shoots, but not significantly, because MI shows greater sensitivity towards cadmium. The maximum cadmium concentration was observed in the EDTA + MI treatment, but Cd uptake was maximum in the VC + MI treatment. The Cd concentration in the shoots increased by 120% in Cd_{EDTA} over the Cd_{100} treatment, followed by Cd_{VC} (65%) and Cd_{FYM} (42%) in the absence of microbial inoculants. The corresponding values in the presence of MI were 107, 51 and 37%, respectively. A similar trend was also observed in the roots in the order $Cd_{EDTA+M} > Cd_{VC+M} > Cd_{FYM+M} > Cd_{100+M}$. MI caused an increase in Cd content of 5.5% in the roots and 4.1% in the shoots in the Cd_{EDTA+M} treatment compared with the Cd_{EDTA} treatment. FYM, VC and EDTA also increased Cd uptake significantly both in the shoots and roots with and without microbial inoculants.

The results indicated that Vermicompost in combination with microbial inoculants is the best treatment for the phytoremediation of Cd-contaminated soil by Indian mustard, as revealed by the Cd uptake values in the shoots: Cd_{VC+M} (2265.7 μg/pot) followed by Cd_{EDTA+M} (2251.2 μg/pot), Cd_{FYM+M} (1485.7 μg/pot) and Cd_{100+M} (993.1 μg/pot).

Key words: phytoremediation, Indian mustard, organic fertilizer, microbial inoculant, Cd uptake

Introduction

The phytoremediation of soil contaminated with heavy metal is an emerging technology that aims to extract or inactivate metals in soils (Salt et al., 1998). Cadmium (Cd) is recognized globally as a hazardous element and is not essential to plants. Cadmium has a wide variety of uses in industry, medicine, dentistry, batteries, science and military applications. The burning of fossil fuels and medical waste accounts for more than 80% of all anthropogenic sources. Reports of the Itai-Itai disease in Japan due to excessive dietary intake of Cd by human beings (Asami, 1981) is one example of heavy metal pollution. The agricultural use of phosphatic fertilizers may also cause the entry of Cd into the human system through crop plants.

In view of the above, there is a need to develop a suitable technique for soil remediation by enhancing the phytoextraction of Cd from contaminated soil. The term phytoremediation is used to describe a system wherein plants, in association with soil organisms, can remove or transform contaminants into harmless and often valuable forms (Chhonkar, 2004). Amongst commercial crops, Indian mustard was found to have a high capacity for extracting and translocating Cd from contaminated soils (Ahmed et al., 2001). Generally, chelate-assisted phytoextraction helps in phytoremediation in two ways: (i) release of bound metals into the soil solution, and (ii) transport of metals to the shoot, which would presumably increase the total metal accumulation in plants (Salt et al., 1998). Synthetic chelators, such as EDTA, DTPA and EGTA, form soluble complexes with metals in the soil and can increase the uptake and translocation of heavy metals through the aboveground tissues (Blaylock et al., 1997). The addition of organic amendments led to higher plant biomass production (Clemente et al., 2005). Vermicompost can be used to remediate metal-contaminated sites because it binds metals and increases uptake (Jadia and Fulekar, 2008). Microbial populations are known to affect heavy metal mobility and availability to the plant through the release of chelating agents, acidification, phosphate solubilization and redox changes, and therefore have potential to enhance phytoremediation processes (Jing et al., 2007). Rhizosphere bacteria can increase the efficiency of Cd phytoremediation by promoting the accumulation of Cd in plants. For better phytoremediation a higher uptake of contaminants by the plants is necessary, which will require a better understanding of effective chelating agents.

The present investigation was undertaken to study the phytoremediation of cadmium-contaminated soil by rhizospheric Indian mustard (*Brassica juncea* L.) with the help of chelating agents and organic manures.

Materials and methods

A pot experiment using sandy loam soil was conducted in a screen-house in the 2007–2008 season. Some selected characteristics of the soil are: pH (1 : 2) 8.2; EC 0.48 dS m^{-1} in water; organic carbon 0.58%; CEC 11.70 cmol (P^+) kg^{-1} soil; total Cd 2.35 μg Cd g^{-1} soil. Earthen pots were lined

with polyethylene to avoid contamination and filled with 20 kg of air-dried soil (< 2 mm). The treatments consisted of Cd (100 μg Cd g^{-1} soil as cadmium chloride), EDTA (2 mmol kg^{-1} soil as disodium salt), farmyard manure (FYM) and vermicompost (VC), 2% by weight each, in all possible combinations. Bioinoculants (*Azotobacter* sp. and *Pseudomonas* sp.) were applied as seed treatment to selected treatment combinations.

A bulk soil sample of the surface layer (0–15 cm) was collected from an untreated field irrigated with sewer water at the vegetable research farm of CCS Haryana Agricultural University, Hisar, India. The soil was air dried, ground and passed through a 2-mm stainless steel sieve to remove gravel and crop residues.

This soil sample was artificially spiked with Cd using $CdCl_2$ as a source of Cd. The bulk soil sample was spread evenly on a polythene sheet placed over the raised platform of the screen-house. A pre-calculated amount of $CdCl_2$ (i.e. 100 mg Cd/300 ml distilled water) was dissolved in distilled water. The solution so prepared was sprinkled over the uniformly spread soil at the rate of 300 ml solution per kg of soil. After sprinkling the solution, the soil sample was covered with a plastic sheet for 48 hours to minimize evaporation and to ensure proper equilibration. Thereafter the cover was taken off and the soil was allowed to dry to a workable moisture content. Then each soil sample was thoroughly mixed, respread uniformly over the plastic sheet and moistened to near field capacity moisture content using distilled water. This cycle was repeated thrice for proper equilibration and the uniform enrichment of the soil with the added Cd. The Cd-enriched bulk soil sample was then air dried and divided into eight equal lots. One lot was kept as a control, the second lot was treated with well decomposed dry farmyard manure (FYM), 2% by weight, and the third with well-decomposed dry vermicompost (VC), 2% by weight, while the fourth lot was given ethylenediaminetetraacetic acid (EDTA) treatment at 2 mmol kg^{-1} soil (0.4 mmol daily for 5 days in 5 split doses) starting at 40 days after sowing (DAS). The remaining four lots were given the same treatments, but were also treated with N_2 fixer (i.e. *Azotobacter* sp.) and phosphorus-solubilising bacteria (i.e. *Pseudomonas* sp.) as seed dressing at the time of sowing. The FYM and vermicompost were mixed uniformly into the Cd-spiked soil one week before sowing.

Indian mustard (*Brassica juncea* L.) was sown as test crop. The basic nutrient requirements of the crop were added in solution form as 50, 50, 60, 10, 5, 5 and 5 mg kg^{-1} soil of N, P, K, Fe, Mn, Zn and Cu, respectively, and mixed thoroughly with the soil before sowing. Each treatment was replicated three times in a completely randomized design. The pots were irrigated to field capacity with deionized water throughout the growth period. Ten seeds of Indian mustard were sown in each pot and thinned to five plants after germination. The plants were harvested 8 weeks after germination at the pre-flowering stage and washed with distilled water. The plant samples were first air dried by keeping them in paper bags and then in an oven at 65 ± 2°C to constant weight. After grinding, 0.5 g dried plant tissue was digested in 20 ml of a concentrated HNO_3:$HClO_4$ (4:1) diacid mixture and the final volume was made up to 25 ml. The cadmium content of the digested solution was determined using an atomic absorption spectrophotometer (GBC 932 plus, Australia).

Results and discussion

Visual toxicity symptoms

The crop plants came under the influence of the toxic concentration of Cd right from the seed germination stage. Starting from the emergence of the plumule up to the harvesting stage of the crop (i.e. at the pre-flowering stage, 56 days after sowing), yellowing of the leaves was observed. In each case, leaf emergence was associated with chlorosis, which started from the margin of the leaves and then progressed consistently inwards. Though the plants continued to grow till the harvesting stage in Cd-enriched soil they remained stunted as compared to those

grown in normal soil. Plants grown in FYM-treated soil had a healthy look compared to the others, which might be attributed to the additional supply of essential nutrients contained in FYM. When the chelating agent EDTA was applied 40 days after sowing, the plants exhibited wilting symptoms a day after application but these disappeared within 2–3 days. This might be attributed to the increased availability of Cd as a Cd–EDTA complex in the soil.

Dry biomass production of plants

The application of EDTA, FYM and VC resulted in a significant increase in dry matter yield in Cd-enriched soil. The highest increase in yield was observed in VC-amended, Cd-enriched soil. This indicates that VC is the most effective agent for the phytoremediation of Cd-contaminated soil. Microbial inoculants had no significant effect on the shoot or root dry matter yield of Indian mustard.

Table 1

Dry matter yield (g pot^{-1}) of shoots and roots of Indian mustard as influenced by different chelating agents and bio-inoculants in Cd-enriched soil

Treatments	Control	Cd_{100}	Cd_{100} + FYM	Cd_{100} + VC	Cd_{100} + EDTA	Mean
Shoots						
(–) Microbial inoculants	34.85	25.61	28.68	39.83	28.51	31.50
(+) Microbial inoculants	35.36	26.43	28.73	39.91	28.84	31.86
Mean	35.11	26.03	28.71	39.87	28.68	
CD (5%)	Microbial inoculants (M) = 0.386; Cd = 0.611; Interaction of M × Cd = 0.864					
Roots						
(–) Microbial inoculants	3.75	2.65	3.81	3.86	2.79	3.37
(+) Microbial inoculants	3.97	2.80	3.96	3.98	2.88	3.52
Mean	3.86	2.72	3.89	3.92	2.84	
CD (5%)	Microbial inoculants (M) = 0.026; Cd = 0.042; Interaction of M × Cd = NS					

NS = non-significant

Cadmium concentration in plants

The application of FYM, VC and EDTA resulted in a significant increase in the Cd concentration in the shoots and roots of Indian mustard as compared to Cd enrichment alone (Cd_{100}). The highest concentration of Cd in Indian mustard plants was observed when Cd-enriched soil was amended with EDTA.

Table 2

Cadmium concentration (μg g^{-1}) in the shoots and roots of Indian mustard as influenced by different chelating agents and bio-inoculants in Cd-enriched soil

Treatments	Control	Cd_{100}	Cd_{100} + FYM	Cd_{100} + VC	Cd_{100} + EDTA	Mean
Shoots						
(–) Microbial inoculants	3.63	34.03	48.43	56.20	75.03	43.47
(+) Microbial inoculants	3.73	37.56	51.70	56.76	78.07	45.57
Mean	3.68	35.80	50.07	56.48	76.55	
CD (5%)	Microbial inoculants (M) = 0.368; Cd = 0.583; Interaction of M × Cd = 0.825					
Roots						
(–) Microbial inoculants	9.76	88.03	103.66	123.83	149.70	95.00
(+) Microbial inoculants	11.83	89.60	104.30	125.66	158.00	97.88
Mean	10.80	88.81	103.98	124.75	153.85	
CD (5%)	Microbial inoculants (M) = 1.498; Cd = 2.368; Interaction of M × Cd = 3.350					

Cadmium uptake in plants

The application of microbial inoculants resulted in a significant increase in Cd uptake in both the shoots and roots. Similarly, the application of FYM, EDTA and VC significantly enhanced the Cd uptake by Indian mustard, VC being the

Table 3

Cadmium uptake (μg pot^{-1}) of shoots and roots of Indian mustard as influenced by different chelating agents and bio-inoculants in Cd-enriched soil

Treatments	Control	Cd_{100}	Cd_{100} + FYM	Cd_{100} + VC	Cd_{100} + EDTA	Mean
Shoots						
(–) Microbial inoculants	126.6	871.6	1381.6	2238.6	2139.0	1351.5
(+) Microbial inoculants	132.0	993.1	1485.7	2265.7	2251.2	1425.6
Mean	129.3	932.4	1433.6	2252.2	2195.1	
CD (5%)	Microbial inoculants (M) = 11.99; Cd = 18.95; Interaction of M × Cd = 26.81					
Roots						
(–) Microbial inoculants	36.7	233.3	395.3	477.9	417.6	312.2
(+) Microbial inoculants	47.0	250.6	413.0	499.7	455.0	333.0
Mean	41.8	241.9	404.2	488.8	436.3	
CD (5%)	Microbial inoculants (M) = 4.06; Cd = 6.42; Interaction of M × Cd = 9.08					

most effective for the removal of Cd from the soil by Indian mustard plants. The most important parameter for phytoremediation is a high uptake of polluting heavy metals into the harvestable biomass. This signifies the necessity of the accumulator species having high biomass yield as well as a higher concentration of the heavy metal. Soluble organics may raise the carrying capacity of the soil solution for trace metals by the formation of soluble organo-metallic complexes (Almas et al., 2000). Wu et al. (2006) also indicated that rhizobacteria are important in the augmentation of metal accumulation in the roots.

Conclusions

The present investigation used performance indicators to reveal that all the soil amendments used in Cd-spiked soil were effective in enhancing the Cd uptake, vermicompost being the most effective. The chemical (EDTA) and organic (FYM, VC) chelates enhanced the Cd concentration in plant shoots and roots. However, EDTA may have environmental consequences. Microbes with or without chelates, FYM and VC proved very effective in promoting both Cd uptake and the tissue Cd concentration. As environment-friendly components of sustainable agriculture, they could be useful both for phytoremediation and as biofertilizers.

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References

Ahmed, K. S., Panwar, B. S., Gupta, S. P. (2001): Phytoremediation of cadmium-contaminated soil by *Brassica species. Acta Agron. Hung.*, **49**, 351–360.

Almas, A. R., Salbu, B., Singh, B. R. (2000): Changes in partitioning of ^{109}Cd and ^{65}Zn in soil as affected by organic matter addition and temperature. *Soil Sci. Soc. Amer. J.*, **64,** 1951–1958.

Asami, T. (1981): *Heavy Metals Pollution in Soils of Japan*. Japan Scientific Societies Press, Tokyo, pp. 257–274.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B. D., Raskin, I. (1997): Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Techno.*, **31,** 860–865.

Chhonkar, P. K. (2004): Phytoremediation: A "Green cure" for heavy metal contaminated soils. *J. Indian Soc. Soil Sc.*, **52,** 357–373.

Clemente, R., Walker, D. J., Bernal, M. P. (2005): Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcóllar (Spain): The effect of soil amendments. *Environ. Pollut.*, **138**, 46–58.

Jadia, C. D., Fulekar, M. H. (2008): Phytoremediation: The application of vermicompost to remove zinc, cadmium, copper, nickel and lead by sunflower plant. *Environ. Engineering Manage. J.,* **7**, 547–558.

Jing, Y., He, Z., Yang, X. (2007): Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *J. Zhejiang Univ. Sci. B,* **8**, 192–207.

Salt, D. E., Smith, R. D., Raskin, I. (1998): Phytoremediation. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **49,** 643–668.

Wu, C. H., Wood, T. K., Mulchandani, A., Chen, W. (2006): Engineering of plant-microbe symbiosis for rhizoremediation of heavy metals. *Appl. Environ. Microbiol.,* **72,** 1129–1134.

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MORPHO-PHYSIOLOGICAL AND NUTRITIONAL CHARACTERIZATION OF RICE BEAN *(Vigna umbellata)*

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Rice bean, a lesser known pulse, has excellent nutritive value. The seed yield of the crop is higher as compared to other pulses of the *Vigna* family. In the present study thirty diverse rice bean genotypes were evaluated for nutritional and morpho-physiological characteristics for selecting overall superior genotypes. Variations were observed for crude protein (16.1–19.12%), carbohydrates (59.28–76.89%), ascorbic acid (0.19–0.80 mg/100 g), crude fibre (4.23–6.0%), limiting amino acids, namely tryptophan (0.85–2.42 g/16 g N) and methionine (0.52–0.67 g/16 g N), and ether extract (0.57–2.13%). Anti-nutritional factors, such as total phenolics, total tannins, condensed tannins, hydrolysable tannins and α-amylase inhibitor, also varied to a considerable extent. The cumulative grading of the genotypes based upon nutritional and morpho-physiological attributes revealed that the genotypes JCR-76, IC-137200, IC-140796 and IC-137189 were nutritionally superior genotypes for consumption.

Key words: rice bean, vigna, phenols, alpha-amylase, antinutritional

Introduction

Food legumes, commonly known as pulses, find an important place in Indian diets as they are good sources of protein and act as an essential supplement for cereal-based meals. Rice bean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi], a less familiar pulse in India, is native to South and South East Asia and is used both as food and fodder. The plants are highly branched, usually bearing bright yellow flowers, present in clusters, and producing large numbers of pods per peduncle. The seed coat colour of rice bean seeds is highly variable, including maroon, green, yellow, brown, light shades of yellowish green, speckled and mottled. This crop is mainly grown in the monsoon season (Agarwal, 2007).

Rice bean has high yield potential and grain yields of up to 2.7 t/ha have been obtained (Anonymous, 1970). Thus, this crop has immense production potential in comparison to other pulse crops (Chaudhari and Prasad, 1972). The nutritional profile of rice bean is also very high, which is mainly attributed to the high content of proteins and essential amino acids, such as tryptophan and methionine, as compared to other traditional pulses (Singh et al., 1980; Sharma et al., 2003). It is rich in protein, calcium, iron and phosphorus (Mal and Joshi, 1991; Shamshad Begum et al., 2004). There is wide variation in the crude protein (14–25%), total digestible protein (0.6–0.8%), tryptophan (0.80–1.10%), methionine (0.39–0.94%), starch (60–67.33%), total soluble sugars (5.0%) and non-reducing sugars (3.71–5.37%) contents (Singh et al., 1980; Srivastava et al., 2001; Saharan et al., 2004). Vitamins like thiamine, riboflavin and niacin are present in appreciable amounts, whereas minerals such as calcium, iron and phosphorus are reported to be present in the order of 450, 10 and 393 mg/100 g, respectively, in rice bean seeds (Sharma et al., 2003). Anti-nutritional factors adversely affect protein digestibility and amino acid availability in the diet (Gilani et al., 2005). The level of anti-nutrients such as phytin phosphorus, polyphenols, saponins and trypsin inhibitors in rice bean is also reported to be low as compared to other food legumes (Khabiruddin et al., 2002; Saharan et al., 2002). To date, no comprehensive studies have been made on the biochemical constituents of this under-utilized crop, particularly involving the germplasm of diverse locations, resulting in inadequate information on the status of biochemical constituents of nutritional significance in this crop. Thus, the present investigation was carried out to evaluate the nutritional attributes and anti-nutritional factors of different rice bean genotypes.

Materials and methods

The experimental material, comprising thirty rice bean genotypes, was sown at the research farm of CSK HPKV, Palampur, Himachal Pradesh, India, in a completely randomized block design (CRBD) with three replications. The genotypes were procured from different locations of the country, including three local germplasm lines (Dhagwar, Panchrukhi and Baroi) and one exotic collection (EC-48223-B from China). The genotype Baroi was used as local check. Each genotype was raised in a single row plot 4 m in length with a spacing of 60 × 10 cm between rows and plants, respectively. Observations were recorded on five randomly tagged plants of each genotype, discarding border plants, for plant height and number of pods per plant. Seed yield was recorded per plot after collecting the pods at different time intervals. Days to 50% flowering and days to 75% maturity were recorded on a plot basis. Analysis of variance was done following Panse and Sukhatme (1984). Oven-dried, finely-ground seed samples were used to determine nutritional parameters. The crude protein content and crude fibre were estimated by standard (AOAC, 1970) methods. Total carbohydrates were estimated by the method given by Dubois et al. (1956). Ascorbic acid content was calculated by the method given by Sadasivam and Balasubramanian (1987), while the limiting amino acids tryptophan and methionine were estimated using the methods of Mertz et al. (1975) and Horn et al. (1946), respectively. Anti-nutrients (phenolics and tannins) were estimated according to Julkunen (1985) and Makkar et al. (1993), respectively. α-Amylase inhibitors were estimated with

the method given by Bernfeld (1955). A cumulative rating was obtained by grading genotypes in descending order for nutritionally desirable characters and in ascending order for anti-nutritional constituents.

Results

Morpho-physiological parameters

Plant height and maturity: The mean plant height of rice bean genotypes varied from 34.1 to 135.67 cm (Table 1), the maximum being observed in the genotype IC-137194 and the minimum in Dhagwar. It is evident from the data that the plant height was significantly higher than the local check variety for all the genotypes, with the exception of Dhagwar, which was significantly shorter than the check. The days to maturity paralleled the days to 50% flowering (Table 1). All three local genotypes (Dhagwar, Panchrukhi and Baroi) flowered and matured early. The exotic collection EC-48223-B (China), IC-140803 (Punjab), IC-137190 (Sikkim) and IC-140805 (Punjab) were late-flowering genotypes and also late in maturity.

Seed yield: Seed yield per plot ranged from 44.0 g in EC-48223-B (China) to 274.27 g in IC-137195 (UP). The lines IC-137195 (UP), IC-019352 (Orissa) and IC-140795 (Punjab) were high-yielding genotypes and were statistically at par with each other (Table 1). These were followed by IC-140796 (Punjab), IC-137194 (UP), IC-140802 (Punjab), IC-140798 (Punjab), IC-137189 (Sikkim), IC-140808 (Punjab) and IC-016789 (Manipur). The lowest seed yield was recorded for EC-48223-B (China) and the local genotypes Baroi and Dhagwar.

The mean values of 100-seed weight ranged from 3.77 g (Local Baroi) to 11.0 g (IC-137194) (Table 1). All the three local germplasm lines had low values for 100-seed weight. The genotype IC-137194 (UP) had a significantly higher value than all the other genotypes. Great variaton was observed in the seed size of rice bean, ranging from large seeds like those of cowpea (IC-137194) to small seeds like those of mung bean (*Vigna mungo*) in the local germplasm (Baroi).

Biochemical parameters

Crude proteins: In mature seeds of rice bean, the crude protein content ranged from 19.12% (Dhagwar) to 16.10% (IC-137186) (Table 2), but the variation was not significant. Other genotypes possessing high crude protein were IC-137189 (18.83), IC-140802 (18.58) and IC-137194 (18.34).

Total soluble carbohydrates: Carbohydrates are important nutritional attributes, as they are a readily available source of energy. The maximum soluble carbohydrate content (76.89%) was observed in the EC-48223-B genotype and the lowest (59.28%) in IC-137195 (Table 2). Two of the thirty genotypes under study (IC-137195 and IC-140802) exhibited significantly lower values of total

Table 1
Variation in morpho-physiological characters among ricebean genotypes

Genotype	Plant height (cm)	Days to 50% flowering	Days to 75% maturity	No. of pods/ plant	Seed yield* g/plot	100-seed weight (g)
IC-137186	70.70	98	146	45	143013	6.77
IC-137187	84.67	99	148	44	122.27	7.23
IC-137188	89.43	95	141	37	167.13	6.90
IC-137189	99.20	98	152	48	182.20	7.97
IC-137190	96.03	105	160	57	94.47	9.37
IC-137191	90.27	103	159	58	117.60	6.60
IC-137194	135.67	97	148	52	199.17	11.00
IC-137195	127.60	98	148	52	274.27	9.93
IC-137199	111.00	98	143	50	150.37	6.87
IC-137200	106.03	98	150	44	160.00	8.03
IC-140795	124.73	96	144	23	259.57	8.10
IC-140796	120.33	103	156	49	199.93	7.23
IC-140798	105.57	101	152	35	184.07	8.17
IC-140802	108.87	100	150	31	185.85	7.43
IC-140803	89.97	105	157	30	102.87	7.93
IC-140804	96.77	100	143	41	142.67	6.47
IC-140805	121.23	104	161	25	146.43	7.17
IC-140808	121.50	99	144	31	177.43	8.23
IC-016771	87.07	93	134	35	123.70	6.87
IC-016789	92.03	98	146	54	176.93	7.37
IC-016801	67.30	95	138	39	103.83	6.23
IC-019352	100.20	93	141	51	268.03	7.03
EC-48223-B	91.83	107	159	45	44.00	9.47
JCR-12	105.53	96	142	55	159.50	7.23
JCR-32	112.47	97	142	58	163.20	8.20
JCR-52	86.77	95	143	35	154.33	6.93
JCR-76	93.80	98	140	58	199.67	6.63
Dhagwar	34.10	90	127	26	67.76	6.17
Panchrukhi	59.53	89	129	31	101.87	5.03
Baroi (Check)	48.73	89	128	27	53.60	3.77
S.E.	2.5	1.33	2.52	–	26.18	0.45
C.D. (%)	7.1	3.77	7.13	NS	74.13	1.27

* plot size 0.6 m × 4.0 m = 2.40 m^2
NS: non-significant

carbohydrates as compared to the local check, while the rest of the genotypes were statistically at par.

Crude fat (Ether extract): The crude fat content (ether extract) is roughly equivalent to the total amount of fat/lipids present in the sample. In rice bean low levels of ether extract were observed in different genotypes, ranging from 0.57% (IC-140796) to 2.13% (IC-137186) (Table 2). Twenty genotypes were observed

Table 2
Variation in nutritional constituents in rice bean genotypes

Genotype	Crude protein (%)	Total carbohydrates (%)	Crude fat (%)	Ascorbic acid (%)	Tryptophan g/16 g N	Methionine g/16 g N
IC-137186	16.10	68.02	2.13	0.186	2.14	0.667
IC-137187	17.72	72.57	1.53	0.341	2.25	0.568
IC-137188	17.94	72.45	1.37	0.325	2.03	0.544
IC-137189	18.83	73.53	1.5	0.488	2.03	0.572
IC-137190	17.8	72.33	1.27	0.457	1.72	0.575
IC-137191	18.03	68.26	1.13	0.472	2.09	0.569
IC-137194	18.34	68.26	1.2	0.356	0.851	0.544
IC-137195	17.57	59.28	1.16	0.326	1.92	0.605
IC-137199	17.38	64.91	0.967	0.333	2.24	0.556
IC-137200	17.77	73.29	1.033	0.457	2.09	0.579
IC-140795	18.33	66.83	0.833	0.697	1.81	0.53
IC-140796	17.38	70.30	0.567	0.658	1.91	0.567
IC-140798	18.22	71.26	1.433	0.697	1.62	0.603
IC-140802	18.58	61.67	1.1	0.689	1.99	0.586
IC-140803	18.22	64.65	1.5	0.79	2.09	0.519
IC-140804	18.19	67.90	1.3	0.442	2.07	0.562
IC-140805	18.33	69.94	1.6	0.441	2.09	0.543
IC-140808	18.22	66.34	1.1	0.534	1.57	0.551
IC-016771	17.83	68.52	1.3	0.434	1.63	0.536
IC-016789	18.05	66.10	1.3	0.418	1.69	0.603
IC-016801	17.74	73.77	1.5	0.798	1.98	0.611
IC-019352	17.18	65.74	0.867	0.476	2.18	0.614
EC-48223-B	17.86	76.89	1.1	0.666	2.42	0.572
JCR-12	17.77	71.14	1.067	0.418	1.88	0.591
JCR-32	17.59	70.54	0.833	0.38	1.58	0.594
JCR-52	17.25	76.77	1.033	0.333	1.61	0.629
JCR-76	16.82	65.98	1.033	0.411	2.25	0.599
Dhagwar	19.12	67.42	1.667	0.264	1.38	0.533
Panchrukhi	17.42	69.34	1.567	0.286	1.57	0.639
Baroi (Check)	17.82	70.42	1.9	0.411	1.74	0.569
S.E.	0.46	2.87	0.16	0.11	0.242	0.287
C.D. (%)	NS	8.13	0.45	0.32	0.686	8.13

NS: non-significant

to be statistically superior to the local check in crude fat content. However, nine genotypes did not differ significantly from the local check in this respect.

Ascorbic acid: The ascorbic acid content in the rice bean genotypes varied from 0.186% (IC-0137186) to 0.798% (IC-016801) (Table 2). In the local check, 0.411% ascorbic acid was recorded. Genotypes IC-140803 and IC-016801 showed significantly higher values for ascorbic acid content, while the rest of the genotypes were statistically at par with the check.

Limiting amino acids (tryptophan and methionine): The rice bean seed genotypes were evaluated for the limiting amino acids tryptophan and methionine. The tryptophan content varied from 0.85 (IC-137194) to 2.42 g/16 g N (EC-48223-B), with a value of 1.74 g/16 g N for the local check (Table 2). The genotype IC-013794 (0.851 g/16 g N) had a significantly lower tryptophan content, whereas the remaining genotypes were statistically at par with the check. The methionine content ranged from 0.52 to 0.67 g/16 g N, with the lowest and highest values for the genotypes IC-140803 and IC-137186, respectively (Table 2). All

Table 3
Variation in anti-nutritional constituents in rice bean genotypes

Genotype	Total phenolics	Simple phenolics	Total tannins	Condensed tannins	Hydrolysable tannins
IC-137186	0.760	0.467	0.347	0.197	0.297
IC-137187	0.732	0.45	0.305	0.028	0.278
IC-137188	0.746	0.411	0.324	0.028	0.296
IC-137189	0.800	0.427	0.352	0.028	0.324
IC-137190	0.680	0.427	0.311	0.039	0.272
IC-137191	0.748	0.589	0.275	0.030	0.297
IC-137194	0.611	0.492	0.275	0.030	0.245
IC-137195	0.632	0.561	0.384	0.029	0.355
IC-137199	0.659	0.472	0.363	0.029	0.333
IC-137200	0.602	0.526	0.377	0.028	0.349
IC-140795	0.655	0.503	0.308	0.029	0.278
IC-140796	0.694	0.431	0.384	0.028	0.355
IC-140798	0.684	0.498	0.421	0.030	0.392
IC-140802	0.680	0.533	0.472	0.030	0.442
IC-140803	0.728	0.416	0.387	0.031	0.356
IC-140804	0.719	0.485	0.37	0.026	0.344
IC-140805	0.682	0.419	0.397	0.030	0.367
IC-140808	0.687	0.471	0.418	0.029	0.389
IC-016771	0.609	0.445	0.33	0.027	0.302
IC-016789	0.649	0.396	0.323	0.028	0.295
IC-016801	0.714	0.415	0.408	0.030	0.377
IC-019352	0.705	0.415	0.418	0.033	0.385
EC-48223-B	0.731	0.344	0.366	0.103	0.263
JCR-12	0.663	0.379	0.309	0.027	0.282
JCR-32	0.723	0.434	0.353	0.049	0.304
JCR-52	0.632	0.462	0.337	0.028	0.309
JCR-76	0.575	0.465	0.288	0.105	0.271
Dhagwar	0.612	0.507	0.382	0.047	0.335
Panchrukhi	0.703	0.536	0.493	0.028	0.464
Baroi (Check)	0.73	0.464	0.363	0.029	0.341
S.E.	0.047	0.052	0.038	0.0039	0.037
C.D. (5%)	NS	NS	0.107	0.011	0.105

NS: non-significant

the genotypes investigated were statistically at par with the local check (0.57 g/16 g N).

Out of the thirty rice bean genotypes, IC-137186, IC-137195, IC-137200, IC-019352 and JCR-76 were observed to contain relatively higher amounts of tryptophan and methionine.

Total and simple phenolics: Phenols, common aromatic compounds with a hydroxyl group, present in variable amounts in all plants, are involved in imparting resistance against diseases and pests, but a high phenol content in food is not nutritionally desirable. The total phenolic content in rice bean seeds varied from 0.575% (JCR-76) to 0.800% (IC-137189) in the present study (Table 3). Genotypes JCR-76 (0.575), IC-137200 (0.602), IC-016771 (0.609) and Dhagwar (0.612) had lower values of total phenolics compared to the local check (0.73), whereas the rest of the genotypes were statistically at par.

The simple phenolics in rice bean seeds ranged from 0.344% (EC-48223-B) to 0.589% (IC-137191). The genotypes IC-137191 (0.589), IC-137195 (0.561) and Panchrukhi (0.536) had the highest values of simple phenolics, whereas EC-48223-B (0.344), JCR-12 (0.379) and IC-016789 (0.396) had the lowest values.

Tannins: The total tannin content in the seeds varied from 0.288 to 0.493%, with minimum and maximum values for IC-137191 and Panchrukhi, respectively (Table 3). The local check had a total tannin content of 0.363%. Genotypes IC-140802 and Panchrukhi had significantly higher values of total tannin content than the check, while the rest of the twenty-seven genotypes were statistically at par with the check. The condensed tannin content varied with the seed coat colour and ranged from 0.026% (IC-140804) to 0.197% (IC-137186) in rice bean seeds.

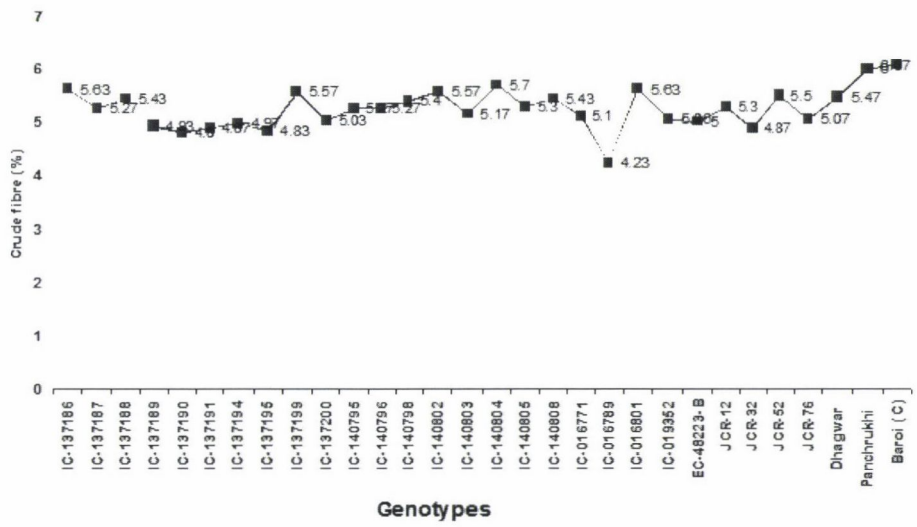

Fig. 1. Crude fibre content in ricebean genotypes

Table 4
Genotypic rating of rice bean for evaluation of overall superior genotypes

Genotype	Seed yield*	Crude protein*	Total carbo-hydrate*	Crude fibre**	Ascorbic acid*	Trypto-phan*	Methio-nine*	Total pheno-lics**	Total tan-nins**	Total	Cumul-ative rating
IC-137186	17	17	18	4	25	5	1	26	11	124	19
IC-137187	19	10	6	9	19	2	8	23	3	99	11
IC-137188	10	8	7	8	22	8	11	24	8	106	13
IC-137189	7	2	4	13	8	8	8	27	12	89	4
IC-137190	24	9	8	15	11	16	7	10	6	106	13
IC-137191	20	7	17	14	10	6	8	25	1	98	10
IC-137194	4	4	17	13	18	24	11	4	1	96	9
IC-137195	1	11	29	15	21	11	4	6	19	117	17
IC-137199	15	13	26	5	20	3	9	8	14	113	16
IC-137200	12	10	5	12	11	6	7	2	17	82	2
IC-140795	3	4	21	9	3	14	12	8	4	88	3
IC-140796	4	13	13	9	6	12	8	14	19	98	10
IC-140798	6	5	9	2	3	18	5	12	24	92	6

Table 4 (cont.)

Genotype	Seed yield*	Crude protein*	Total carbo-hydrate*	Crude fibre**	Ascorbic acid*	Trypto-phan*	Methio-nine*	Total pheno-lics**	Total tan-nins**	Total	Cumul-ative rating
IC-140802	5	3	28	3	4	9	6	10	25	93	7
IC-140803	22	5	27	10	2	6	13	20	20	125	20
IC-140804	18	6	19	3	12	7	9	18	16	108	14
IC-140805	16	4	14	9	13	6	11	10	21	104	12
IC-140808	8	5	22	8	7	22	10	13	23	108	14
IC-016771	17	9	16	11	14	19	11	3	9	109	15
IC-016789	9	7	23	16	15	17	5	7	7	106	13
IC-016801	21	10	3	4	1	10	4	17	22	92	6
IC-019352	2	15	25	11	8	4	4	16	23	108	14
EC-48223-B	26	9	1	12	5	1	8	22	15	99	11
JCR-12	13	10	10	9	15	13	6	9	5	90	5
JCR-32	11	11	11	14	17	21	6	19	13	124	19
JCR-52	14	14	2	6	20	20	3	6	10	95	8
JCR-76	4	16	24	11	16	2	5	1	2	81	1
Dhagwar	25	1	20	7	24	23	12	5	18	135	21
Panchrukhi	23	12	15	2	23	22	2	3	26	139	22
Baroi (C)	26	9	12	1	16	15	8	15	14	122	18

* Genotypes graded in descending order for nutritionally desirable characters
** Genotypes graded in ascending order for nutritionally undesirable constituents

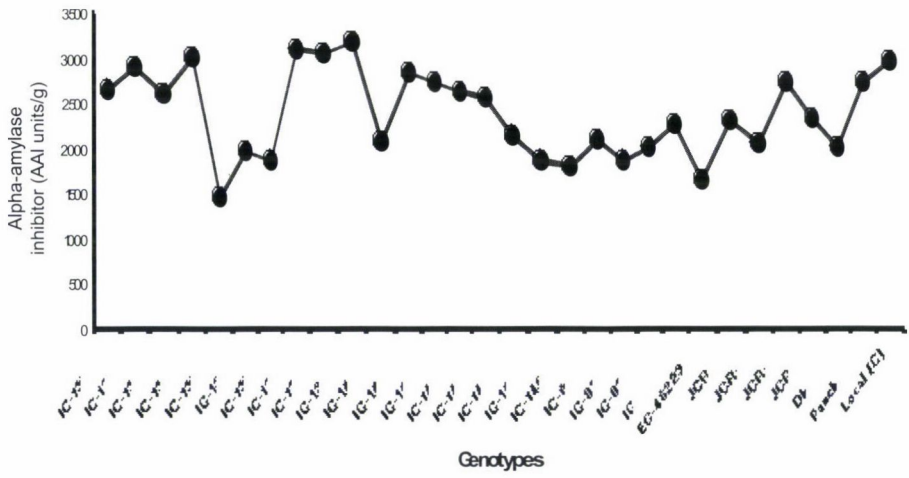

Fig. 2. α-Amylase inhibitor content in different rice bean genotypes

Three genotypes (IC-137186, JCR-76 and EC-48223-B) showed significantly higher values of condensed tannin content as compared to the check.

The remaining genotypes were statistically at par with the local check. The mean values of hydrolysable tannin (Table 3) varied from 0.245% (IC-137194) to 0.464% (Panchrukhi). The genotypes IC-140802 (0.442%) and Panchrukhi (0.464%) showed significantly higher values of hydrolysable tannin than the local check (0.341%), while genotypes IC-137194 (0.245%), EC-48223-B (0.263%) and JCR-76 (0.271%) had lower values.

Crude fibre: The crude fibre content in rice bean genotypes varied from 4.23% (IC-016789) to 6.07% (local check: Baroi), the highest value recorded (Fig. 1). Most of the genotypes were statistically at par with the local check (Baroi) for crude fibre content.

α-Amylase inhibitor: α-Amylase inhibitors are considered as starch blockers due to their mode of inhibitory action. The mean values of α-amylase inhibitor (Fig. 2) ranged from 1469 (in IC-137190) to 3180 AAI units/g (in IC-137200). Genotypes IC-137200 (3180.03), IC-137195 (3097.50), IC-137199 (3051.30), IC-137189 (3008.77) and the local check (2978.70) were found to have high α-amylase inhibitor activity, resulting in better storage characteristics. The lowest activity of α-amylase inhibitors was recorded in the genotypes IC-137190 (1469.10) and EC-48223-B (1662.10).

The cumulative rating of rice bean genotypes carried out on the basis of overall performance in respect of productivity and nutritional and anti-nutritional profiles revealed that the genotypes JCR-76, IC-137200, IC-140796 and IC-137189 were high-yielding, nutritionally superior genotypes (Table 4).

Discussion

Grain legumes are the major source of dietary proteins in all the developing countries. Rice bean is a legume with rich genetic diversity and high agricultural and nutritional potential and has the additional advantage of being able to grow well in comparatively poor soils in hot and humid climates and of having resistance to storage pests and serious diseases. In the present study the local genotypes (Dhagwar, Panchrukhi and Baroi) were early maturing. Genotypes EC-48223-B (China), IC-140803 (Punjab), IC-137190 (Sikkim) and IC-140805 (Punjab) were late flowering genotypes and were also late in maturity. The lowest seed yield was recorded in genotype EC-48223-B (China). High crude protein was recorded in the genotypes IC-137189 (18.83%), IC-140802 (18.58%) and IC-137194 (18.34%). The maximum soluble carbohydrate content was observed in genotype EC-48223-B. IC-137186, IC-137195, IC-137200, IC-019352 and JCR-76 were observed to contain relatively higher amounts of tryptophan and methionine than the other genotypes. Genotype IC-140802 was not only superior for crude protein content, but also exhibited higher contents of both the essential amino acids than the local check. Despite being good, cheap sources of plant protein, vitamins, minerals and fibre, legumes also possess factors such as phenolic compounds, which are known to inhibit the activity of digestive enzymes like α-amylase, trypsin, chymotrypsin and lipase, besides decreasing the digestibility of proteins and carbohydrates and the availability of vitamins and minerals. Among the thirty genotypes, JCR-76 (0.575), IC-137200 (0.602), IC-016771 (0.609) and Dhagwar (0.612) had lower values of total phenolics than the local check (0.73). Genotypes IC-137194 (0.245%), EC-48223-B (0.263%) and JCR-76 (0.271%) had the lowest values of condensed tannins. The cumulative rating of the genotypes revealed that JCR-76, IC-137200, IC-140796 and IC-137189 were best overall, being nutritionally excellent and high yielding, making them suitable for large-scale consumption as nutritionally rich, comparatively low cost pulses. As the majority of the population suffer from a shortage of nutritional sources, research efforts need to be concentrated on high-yielding, nutritionally rich sources of protein like rice bean, in order to exploit their full potential as an accessible source of nutrients.

References

Agarwal, K. P. (2007): *Technologies from Mission Mode Research, 1995–2005.* Indian Council of Agricultural Research, Pusa, New Delhi, India, pp. 20–21.

Anonymous (1970): *Final Technical Report, PL 480.* Scheme on "Collection, maintenance and assessment of indigenous genetic stocks of grasses and legumes for food, forage and conservation purposes." Division of Plant Introduction, IARI, New Delhi, India.

AOAC (1970): *Official Methods of Analysis.* 11th edition. Association of Official Analytical Chemists, Washington, DC.

Bernfeld, P. (1955): Amylases, α and β. *Method. Enzymol.,* **1,** 149–158.

Chaudhari, A. P., Prasad, B. (1972): Flowering behaviour and yield of ricebean (*Phaseolus calcaratus* Roxb.) in relation to dates of sowing. *Indian J. Agric. Sci.*, **42**, 627–630.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956): Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–356.

Gilani, G. S., Cockell, K. A., Sepehr, E. (2005): Effects of anti-nutritional factors on protein digestibility and amino acid availability in foods. *J. AOAC Int.*, **88**, 967–987.

Horn, M. J., Jones, D. B., Blum, A. E. (1946): Colorimetric determination of methionine in proteins and foods. *J. Biol. Chem.*, **116**, 313–320.

Julkunen, T. R. (1985): Phenolics constituents in the beans of northern willows. Methods for the analysis of certain phenolics. *J. Agric. Food Chem.*, **33**, 213–217.

Khabiruddin, M., Gupta, S. N., Tyagi, C. S. (2002): Nutritional composition of some improved genotypes of rice bean *(Vigna umbellata)*. *Forage Res.*, **28**, 104–105.

Makkar, H. P. S., Blummel, M., Borowy, N. K., Becker, K. (1993): Gravimetric determination of tannins and their correlation with chemical and protein precipitation methods. *J. Sci. Food Agric.*, **61**, 161–165.

Mal, B., Joshi, V. (1991): Under-utilized plant resources. In: Paroda, R. S., Arora, R. K. (eds.), *Plant Genetic Resources Conservation and Management Concepts and Approaches*. International Board for Plant Genetic Resources, Pusa, New Delhi, India.

Mertz, E. T., Jambunathan, R., Mishra, P. S. (1975): Protein quality. *Agricultural Research Bull.* No. 70, Purdue University, USA, p. 9.

Panse, V. G., Sukhatme, P. V. (1984): *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi, 381 pp.

Sadasivam, S., Balasubramanian, T. (1987): *Practical Manual in Biochemistry*. Tamilnadu Agricultural University, Coimbatore, p. 14.

Saharan, K., Khetarpaul, N., Bisnoi, S. (2002): Variability in physico-chemical properties and nutrient composition of newly released rice bean and faba bean cultivars. *J. Food Compos. Anal.*, **15**, 159–167.

Saharan, K., Khetarpaul, N., Bisnoi, S. (2004): Contents and digestibility of carbohydrates of rice bean and faba bean as affected by simple inexpensive processing methods. *Nutr. Food Sci.*, **30**, 13–16.

Shamshad Begum, S., Musharati Begum, J., Annapurna, M. L. (2004): Utilization of rice bean products in the daily diet. *J. Dairying Foods and Home Sci.*, **23**, 137–142.

Sharma, B. K., Das, A., Banjarbarnah, K. M. (2003): Underutilized life support crop species: Production and research in north-eastern hill region of India. *Household Food and National Security Programme 1*. NATP.

Singh, S. P., Mishra, B. K., Chandel, K. P. S., Pant, K. C. (1980): Major food constituents of rice-bean *(Vigna umbellata)*. *J. Food Sci. Tech.*, **17**, 238–240.

Srivastava, R. P., Srivastava, G. K., Gupta, R. K. (2001): Nutritional quality of rice bean *(Vigna umbellata)*. *Indian J. Agric. Biochem.*, **14**, 55–56.

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SWEET POTATO (*Ipomoea batatas* L.)-BASED STRIP INTERCROPPING: I. INTERSPECIFIC INTERACTIONS AND YIELD ADVANTAGE

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A field experiment was conducted at the Regional Centre of the Central Tuber Crops Research Institute, Dumduma, Bhubaneswar for three consecutive years (2006–2008) under rainfed conditions on Alfisols to quantify the effects of strip intercropping on crop yields and yield components. A significantly higher yield was obtained from sweet potato (*Ipomoea batatas* L.) border rows when pigeonpea (*Cajanus cajan* (L.) Millsp.) was intercropped. Analyses of sweet potato yield components indicated that the number of roots/plant, root length and root diameter were significantly higher in border rows when rice (*Oryza sativa* L.), finger millet (*Eleusine coracana* L.) and pigeonpea were used as intercrop compared to monoculture sweet potato. The number of tubers/plant of sweet potato in border rows was significantly lower when maize (*Zea mays* L.) was intercropped, but the root length and root diameter were found to increase compared to sole sweet potato. The yields of rice, finger millet, maize and pigeonpea in inside rows in strip cropping were a little higher than in monoculture. The yield difference was mainly due to an increase in the number of seeds/panicle or cob. Sweet potato was the dominant crop when grown with rice or finger millet, but it was the subordinate crop when grown along with maize or pigeonpea. Sweet potato yields were consistently higher in strip intercropping than in monoculture when calculated across all the strips on an equal area basis. A strip intercropping system involving sweet potato + pigeonpea resulted in a higher land equivalent ratio (1.31) and net return ($623.9) compared to the other forms of intercropping and to monocropping.

Key words: aggressivity, maize, pigeonpea, finger millet, rice, strip intercropping, sweet potato

Introduction

Intercropping or mixed cropping is a predominant production system in small land holdings in Latin America (Graham and Vance, 2000), Africa (Graham and Vance, 2000) and Southern Asia (Rerkasem et al., 1988). Intercropping is

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gaining importance because it not only provides biological insurance against risks in the case of aberrant rainfall behaviour in a dry land environment (Dutta and Bandyopadhyay, 2006), but also more labour employment (Nedunchezhiyan et al., 2008), as well as some insect and disease control (Stinner and Blair, 1990). In uplands, intercropping and crop substitution stabilize crop yields (Rao et al., 1982). When crops of different growth habits are put together in an intercropping system, it provides greater opportunity to secure higher yields from the same piece of land (Sarkar and Pal, 2004). Among the component crops, competition is minimal when the difference in growth duration is wider in areas having a long crop-growing period (Amede, 1995). The synergetic effect of the component crops during intercropping has also been reported.

Sweet potato (*Ipomoea batatas* L.) is grown for its tuberous root, which is a source of carbohydrate. It is known for drought tolerance. Mid-season and terminal droughts may reduce the sweet potato yield, but complete crop failure is unlikely. The intercropping of sweet potato with cereals, millets and pulses could act as a contingency crop and increase land use efficiency, apart from augmenting farm yields in upland rainfed conditions. Intercropping sweet potato between maize (*Zea mays* L.) rows is practised not only in regions with intermittent drought and relatively longer growing periods, but also in areas with frequent terminal drought (Amede, 2001).

Sweet potato is generally planted using the ridge and furrow method, whereas cereals and pulses are sown in flat beds. A strip intercropping system involving maize and soybean (*Glycine max* (L.) Merrill) has the potential to reduce soil erosion (Lesoing and Francis, 1999a) and weed density (Glowaeka, 2007) and to increase biological and economic efficiency (Lesoing and Francis, 1999b).

No information is available on the yield and yield components of sweet potato and other intercrops when grown in strip cropping. If yield components and the spatial cropping patterns that influence their yield contributions can be identified, systems can be designed to increase potential productivity. Hence the present investigation was carried out to find out the effects of row position on the yields and yield components of sweet potato, rice (*Oryza sativa* L.), finger millet (*Eleusine coracana* L.), maize and pigeonpea (*Cajanus cajan* (L.) Millsp.) and the relative timing of competition for growth resources in strip intercropping compared with a monoculture.

Materials and methods

The field experiment was conducted at the Regional Centre of the Central Tuber Crops Research Institute (20°14'50" N and 85°47'06" E), Dumduma, Bhubaneswar in three consecutive years (2006–2008) under rainfed conditions on Alfisols. The experiment was laid out in a randomized block design (RBD) with three replications. The treatments consisted of five sole crops, namely sweet potato, rice, finger millet, maize and pigeonpea, and four strip intercropping systems (1.8 m

strip for each component crop): sweet potato + rice (3:9 rows), sweet potato + finger millet (3:9 rows), sweet potato + maize (3:3 rows), sweet potato + pigeonpea (3:3 rows). The varieties Kishan (sweet potato), Vandana (rice), Nilachal (finger millet), Navjot (maize) and UPAS 120 (pigeonpea) were used in the experiment. The sweet potato, rice, finger millet, maize and pigeonpea crops were sown/planted at spacings of 60 × 20 cm, 20 × 15 cm, 20 × 15 cm, 60 × 30 cm and 60 × 20 cm, respectively. Sweet potato was planted using the ridge and furrow method, whereas the other crops were sown in flat beds. The recommended doses of fertilizer $N:P_2O_5:K_2O$ for sole crops of sweet potato, rice, finger millet, maize and pigeonpea were 50:25:50, 40:40:40, 30:30:40, 60:60:60 and 40:40:20 kg/ha, respectively. In strip intercropping each component crop occupies 50% of the area, hence 50% of the recommended dose of fertilizers was applied to each crop. The full dose of phosphorus and half the dose of nitrogen and potassium were applied as basal fertilizer at the time of sowing/planting. The remaining half dose of nitrogen and potassium was applied 30 days after sowing/planting to all the crops. Recommended practices were followed for all the crops. The crops were sown/planted on 21 June 2006, 22 June 2007 and 15 June 2008. Rice, maize, finger millet, sweet potato and pigeonpea were harvested 85, 90, 105, 120 and 210 days after sowing/planting.

The rainfall during the crop years 2006–2008 ranged from 1316.7 to 1397.5 mm, on 70–74 rainy days. The average maximum temperature ranged from 29.2–37.5°C and the average minimum temperature from 14.6–26.5°C. July and August received the highest quantities of rain. The mean relative humidity ranged from 62 to 88%. The climate of the region is characterized by hot, humid summers and cold, dry winters. The soil had pH 5.4, 242 kg/ha available N, 18.6 kg/ha available P and 158 kg/ha available K before the start of the experiment. The water-holding capacity of the soil was 12.2%.

Aggressivity, a simple measure of how the relative yield increase in crop *a* compares with that of crop *b* in an intercropping system, was calculated for border rows, middle rows and for the whole system. As both the species were sown in equal proportions as regards area in the strip intercropping, the aggressivity was calculated using the following equation:

$$Aab = (Yab/Yaa) - (Yba/Ybb)$$

where *Yaa* and *Ybb* are yields as sole crops of *a* and *b,* and *Yab* and *Yba* are yields as intercrops of *a* and *b*.

If *Aab* = 0, both crops are equally competitive; if *Aab* is positive, *a* is dominant; if *Aab* is negative, *a* is the subordinate crop.

The land equivalent ratio (LER), an accurate assessment of the biological efficiency of the intercropping system, was calculated as

$$LER = (Yab/Yaa) + (Yba/Ybb).$$

Values of LER greater than 1 are considered advantageous.

The sweet potato equivalent yield was calculated by converting the yields of the intercrops to the yield of sweet potato on the basis of the prevailing market price of each crop. The economics of different crops and crop combinations were computed on the basis of the prevailing market rates for produce and agro inputs.

The data of each crop season were statistically analysed separately using Genstat software. Then the homogeneity of error variance was tested using Bartlett's χ^2-test. As the error variance was homogeneous, pooled analysis was done. The data collected were subjected to analysis of variance (ANOVA) using Genstat software. Comparison of treatment means for significance at 5% was done using the least significant difference (LSD) method.

Results and discussion

Sweet potato yield and yield components

Sweet potato border rows in strip intercropping gave a higher root yield than sole crop rows (Table 1). The sweet potato plant yield in border rows was 32.9, 38.9, 10.2 and 44.3% higher than the sole sweet potato plant yield when strip intercropped with rice, finger millet, maize and pigeonpea, respectively. One of the main reasons for higher yields in border rows of strip cropping is that the component crops are able to use growth resources rationally and make better use of natural resources than rows in a monoculture. Also, interspecies interference was minimal, while the complementary use of other growth resources was maximized in border rows. Cropping systems that include legumes such as pigeonpea *(Cajanus cajan)* or white lupin can enhance P availability to associated species (Ae et al., 1990; Snapp, 1998). The growth of legumes can result in P release exceeding the requirement of the legume, with the remainder available to the associated crop (Graham and Vance, 2000). The sweet potato plant yield in middle rows was 5.4, 7.8, 0.6 and 10.8% higher than sole sweet potato, when strip intercropped with rice, finger millet, maize and pigeonpea, respectively. A drastic reduction in the interspecific interaction effect was noticed towards the middle row as the sweet potato rows were widely spaced (60 cm). However, the mean root yield/plant of sweet potato considering the whole strip was 19.2, 23.4, 5.4 and 27.5% higher when intercropped with rice, finger millet, maize and pigeonpea than for monoculture sweet potato. A significantly higher root yield/plant of sweet potato was recorded in border rows in strip intercropping, being 31.7% higher than for monoculture sweet potato (Table 2). The sweet potato yield in the inside rows of strip cropping was also higher than in a monoculture, but was statistically comparable.

An analysis of sweet potato root yield components indicated that the number of roots/plant, root length and root diameter were significantly higher in border rows when rice, finger millet or pigeonpea was the intercrop, compared to monoculture sweet potato (Table 1). The number of roots/plant of sweet potato in border rows was significantly lower (7.4%) when maize was intercropped, but the root length (4.3%) and root diameter (14.6%) increased compared to sole sweet potato. The number of roots/plant is decided in sweet potato within 40 days of planting (Nedunchezhiyan et al., 2004). Maize, a vigourously growing crop, competed for resources with sweet potato and suppressed it at an early stage. Competition for moisture or nutrients was reported to contribute to the lower border row yield and seed number of soybean observed in maize-soybean strip intercropping (Lesoing and Francis, 1999a). Once the maize was harvested (90 days after sowing), the sweet potato fully utilized the available resources. A significantly higher number of roots/plant, root length and root diameter were observed for sweet potato in the border rows of strip intercropping with values 11.1, 9.5 and 12.1% higher than sole sweet potato (Table 2). Middle row yield components, such as

Table 1
Yield components and yield of sweet potato in intercropping and sole cropping systems (pooled data of 3 years)

Cropping system	No. of roots/plant			Root length (cm)			Root diameter (cm)			Root yield/plant (g)		
	Border row	Middle row	Mean	Border row	Middle row	Mean	Border row	Middle row	Mean	Border row	Middle row	Mean
Sole cropping	2.7	2.7	2.7	11.6	11.6	11.6	8.2	8.2	8.2	167	167	167
Sweet potato + rice	2.9	2.7	2.8	12.4	11.9	12.1	8.9	8.3	8.6	222	176	199
Sweet potato + finger millet	3.2	2.8	3.0	12.9	12.0	12.4	9.1	8.3	8.7	232	180	206
Sweet potato + maize	2.5	2.7	2.6	12.1	11.9	12.0	9.4	8.4	8.9	184	168	176
Sweet potato + pigeonpea	3.3	2.9	3.1	13.4	11.9	12.6	9.4	8.4	8.9	241	185	213
LSD ($P = 0.05$)	0.1	0.1	0.1	0.5	0.4	0.3	0.3	0.1	0.2	13	9	11

Table 2
Yield and yield components of sweet potato in various row positions (pooled data of 3 years)

Row position	No. of roots/plant	Root length (cm)	Root diameter (cm)	Root yield/plant (g)
Monocropping	2.7	11.6	8.2	167
Strip intercropping				
Border row	3.0	12.7	9.2	220
Middle row	2.8	11.9	8.4	177
Mean row	2.9	12.3	8.8	199
LSD ($P = 0.05$)	0.2	0.5	0.6	11

number of roots/plant, root length and root diameter, were somewhat higher but statistically at par with monoculture sweet potato.

Rice, finger millet, maize and pigeonpea yield and yield components

The rice, finger millet, maize and pigeonpea yields were higher in strip intercropping with sweet potato than in a monoculture (Table 3). The increase in yield was due to the higher yield in border rows. Pigeonpea, maize, finger millet and rice border rows may have a yield advantage owing to their greater height compared with the adjacent sweet potato rows, and may also have a competitive advantage in the root zone. In rice and finger millet the border row effect was found to a decreasing extent up to three rows towards the centre due to the closer spacing (data not presented). Yield components such as number of panicles or pods/plant and seeds/panicle, cob or pod were significantly higher than for the sole crop. Increased panicles or pods/plant and seeds/panicle, cob or pod may have been due to greater light interception by border rows, resulting in higher photosynthesis rates and the development of more productive tillers and pods. However, the number of cobs/plant remained the same in both strip and sole cropping. This may be due to the genetic character of the variety. No difference in the seed weight of rice/finger millet/maize/pigeonpea was observed between strip and sole cropping. Harper (1961) attributed this to internal or physiological homeostasis with respect to the organ that is essential for reproduction and dispersal. Egli and Yu (1991), Lesoing and Francis (1999a) and Pavlish (1989) also reported that the seed weight and size of maize, soybean and grain sorghum were not affected by strip intercropping (maize–soybean and grain sorghum–soybean).

The inside row yields of rice, finger millet, maize and pigeonpea in strip cropping were a little higher than in a monoculture (Table 3). The yield difference was mainly due to an increase in the number of seeds/panicle or cob. The number of panicles/plant and cobs/plant was not affected in the inside rows of strip cropping compared to sole cropping. However, a 5.1% increase in the number of pods/plant was observed in the inside rows of pigeonpea compared to a monoculture. The number of seeds/pod remained the same in border rows as in sole crop rows of pigeonpea. The yield difference between inside rows and monoculture in rice and finger millet was negligible compared to that of maize and pigeonpea. There were seven inside rows in strip intercropped rice and finger millet due to the closer spacing. Though the rows adjacent to the border rows gave higher yields, the difference decreased towards the centre (data not presented). In the case of maize or pigeonpea, the number of inside rows was only one.

Table 3
Yield components and yield of rice, finger millet, maize and pigeonpea in intercropping and sole cropping systems (pooled data of 3 years)

Cropping system	Rice/Finger millet/Maize/Pigeonpea															
	Panicles/plant, cobs/plant or pods/plant				Grains/panicle, cob or pod				1000-seed weight (g)				Seed yield/plant (g)			
	Intercrop			Sole	Intercrop			Sole	Intercrop			Sole	Intercrop			Sole
	B	Mi	Me		B	Mi	Me		B	Mi	Me		B	Mi	Me	
Rice	–	–	–	5.3	–	–	–	80.7	–	–	–	20.80	–	–	–	8.9
Finger millet	–	–	–	2.8	–	–	–	1178.0	–	–	–	1.91	–	–	–6.3	
Maize	–	–	–	1.0	–	–	–	199.0	–	–	–	232.00	–	–	–	46.1
Pigeonpea	–	–	–	76.2	–	–	–	3.8	–	–	–	83.20	–	–	–	24.1
Sweet potato + rice	5.5	5.3	5.4	–	92.8	83.4	88.1	–	20.80	20.80	20.80	–	10.7	9.1	9.9	–
Sweet potato + finger millet	2.8	2.8	2.8	–	1280.0	1188.0	1234.0	–	1.91	1.91	1.91	–	6.7	6.5	6.6	–
Sweet potato + maize	1.0	1.0	1.0	–	229.0	205.0	217.0	–	234.00	232.00	233.00	–	52.6	48.2	50.4	–
Sweet potato + pigeonpea	117.9	80.1	99.0	–	4.0	3.8	3.9	–	83.50	83.30	83.40	–	37.1	27.3	32.2	–

B: Border row; Mi: Middle row; Me: Mean

Table 4
Aggressivity of sweet potato-based strip intercropping systems

Strip intercropping	Aggressivity		
	Border rows	Middle rows	Mean
Sweet potato + rice	0.11	0.03	0.08
Sweet potato + finger millet	0.33	0.05	0.17
Sweet potato + maize	(–) 0.04	(–) 0.05	(–) 0.04
Sweet potato + pigeonpea	(–) 0.10	(–) 0.02	(–) 0.06

Aggressivity

The aggressivity of border rows, middle rows and mean (over all), presented in Table 4, revealed that sweet potato was the dominant crop when grown with rice or finger millet. However, it was the subordinate crop when grown with maize or pigeonpea. When intercrop rows are placed sufficiently widely and the recommended dose of fertilizers is applied based on area occupation, there is no competition for light and nutrients. However the complementary effect depends on the crop species. In sweet potato + rice and sweet potato + finger millet strip intercropping sweet potato exhibited a far greater complementary effect than rice or finger millet. The greater aggressivity of maize in sweet potato + maize intercropping might be due to the height and rapid growth of maize. Similarly, the long duration of pigeonpea, which utilized all the available resources after the harvest of sweet potato, might be responsible for its higher aggressivity index. The aggressivity index was higher in border rows than in middle rows (Table 4). The interspecies interaction decreased when moving away from the border.

Yield advantages

The yield advantage of a system was evaluated in three ways based on crop yields, LER and net return per hectare, as shown in Table 5. Sweet potato yields were consistently higher (5.4 to 27.7%) in strip intercropping than in monoculture when calculated across the entire strip on an equal area basis. The highest value was obtained for pigeonpea (27.7%) and the lowest for maize (5.4%). The rice, finger millet, maize and pigeonpea yields were higher (10.0, 4.7, 9.3 and 33.0%) in the strips than in monoculture (Table 5). The significant differences between inside and border row positions are reduced in the evaluation of whole strips. The interspecific complementarity effect was found to be low in finger millet, whereas it was higher in pigeonpea. This might be due to genetic character in the former case and to long duration in the latter case. The root equivalent yield of sweet potato strip intercropping with rice, finger millet, maize and pigeonpea was higher than for sole rice, finger millet, maize and pigeonpea. The sweet potato + pigeonpea intercropping system had a significantly higher root equivalent yield

Table 5
Root equivalent yield and economics of sweet potato-based strip intercropping systems (pooled data of 3 years)

Cropping system	Root yield (kg/ha)	Seed yield (kg/ha)	Root equivalent yield (kg/ha)	LER	Net return ($/ha)
Sole cropping					
Sweet potato	13367	–	13367	–	484.9
Rice	–	2532	4220	–	89.1
Finger millet	–	1778	2963	–	22.0
Maize	–	2395	3991	–	74.1
Pigeonpea	–	1877	7508	–	335.3
Strip intercropping					
Sweet potato + rice	7942	1392	10262	1.14	377.1
Sweet potato + finger millet	8221	931	9751	1.14	363.1
Sweet potato + maize	7043	1309	9224	1.08	317.9
Sweet potato + pigeonpea	8538	1249	13534	1.31	623.9
LSD ($P = 0.05$)	–	–	403	–	–

Selling price: Sweet potato $0.06/kg; rice/finger millet/maize $0.11/kg; pigeonpea $0.26/kg
LER: Land equivalent ratio

(13,538 kg/ha) compared to the other cropping systems, followed by sweet potato sole cropping (13,367 kg/ha) (Table 5).

The LER gives an accurate assessment of the biological efficiency of the intercropping situation. The results of the present experiment showed crop complementarity and yield advantages in all intercropping system as the LER values were greater than unity. The strip intercropping system involving sweet potato + pigeonpea resulted in the highest LER (1.31), indicating the greater biological efficiency of the system. The monetary advantage based on the tuber equivalent yield also indicated the superior economic viability of the sweet potato + pigeonpea intercropping (Table 5). The highest net return ($623.9) was registered for sweet potato + pigeonpea, followed by sweet potato sole cropping ($484.9).

Conclusions

The yields and yield components of sweet potato were found to be affected by strip intercropping. The sweet potato border rows in strip intercropping yielded significantly better than sole crop rows, especially when pigeonpea was intercropped. Maize as an intercrop also increased the yield of sweet potato border rows compared to monoculture sweet potato. Interspecies interference was minimal, while the complementary use of other growth resources was maximized in the border rows. An analysis of the sweet potato yield components indicated that the number of tubers/plant, tuber length and tuber diameter were significantly higher in border rows. The seed number exhibited significant differences between row positions more often than the seed weight, suggesting that the reproductive period is more important in terms of competition for growth resources. To further exploit this advantage, the plant density could be increased in the border rows, with the possible use of narrower strips (e.g. alternating two-row strips), to increase the yields in the strip borders.

References

Ae, N., Arihara, J., Okada, K., Yoshihara, T., Johansen, C. (1990): Phosphorus uptake by pigeon pea and its role in cropping systems of the Indian subcontinent. *Science,* **248**, 477–480.

Amede, T. (1995): Yield gain and risk minimization in maize through cultivar mixtures in the semi-arid zones of the Rift Valley, Ethiopia. *Expl. Agric.*, **31**, 161–168.

Amede, T. (2001): Interaction of components of sweet potato-maize intercropping under the semi-arid conditions of the Rift Valley, Ethiopia. *Trop. Agric. (Trinidad),* **78**, 1–7.

Dutta, D., Bandyopadhyay, P. (2006): Production potential of intercropping of groundnut (*Arachis hypogaea*) with pigeonpea (*Cajanus cajan*) and maize (*Zea mays*) under various row proportions in rainfed Alfisols of West Bengal. *Indian J. Agron.,* **51**, 103–106.

Egli, D. B., Yu, Z. W. (1991): Crop growth rate and seeds per unit area in soybeans. *Crop Sci.*, **31**, 439–442.

Glowaeka, A. (2007): Effect of strip intercropping system on weed infestation in maize. *Acta Agrophysica*, **10**, 573–582.

Graham, P. H., Vance, C. P. (2000): Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Res.*, **65,** 93–106.

Harper, J. L. (1961): Approaches to the study of plant competition. pp. 1–39. In: Milthrope, F. L. (ed.), *Mechanisms in Biological Competition*. 15th Symposium of Society of Experimental Biology. Cambridge Univ. Press, Cambridge.

Lesoing, G. W., Francis, C. A. (1999a): Strip intercropping effects on yield and yield components of corn, grain sorghum and soybean. *Agron. J.*, **91**, 807–813.

Lesoing, G. W., Francis, C. A. (1999b): Strip intercropping of corn-soybean in irrigated and rainfed environments. *J. Production Agric.*, **12**, 187–192.

Nedunchezhiyan, M., Byju, G., Naskar, S. K. (2008): Yield potential and economics of elephant foot yam *(Amorphophallus paeoniifolius)* + greengram (*Vigna radiate*) intercropping system as influenced by mulching and fertilizer levels. *Indian J. Agric. Sci.*, **78**, 17–20.

Nedunchezhiyan, M., Srinivasulu Reddy, D., Suryaprakasa Rao, J. (2004): Characteristics of dry matter production and partitioning in sweet potato. pp. 110–115. In: Reddy, P. V. (ed.), *Proceedings of National Seminar on Physiological Interventions for Improved Crop Productivity and Quality: Opportunities and Constraints.* Regional Agricultural Research Station, Tirupati, India.

Pavlish, L. A. (1989): *Influence of intercropping patterns on yield of sorghum and soybeans*. M.Sc. Thesis, Univ. Nebraska, Lincoln.

Rao, M. V., Jha, K. P., Moorthy, B. T. S., Mondal, B. K. (1982): Intercropping green gram and groundnut with rice and finger millet in the winter season and the feasibility of second crop in rainfed uplands of coastal Orissa. *Indian J. Agric. Sci.*, **52**, 657–664.

Rerkasem, B., Rerkasem, K., Peoples, M. B., Herridge, D. F., Bergersen, F. J. (1988): Measurement of N_2 fixation in maize (*Zea mays* L.) – ricebean (*Vigna umbellata* Thunb.), Ohwi and Ohashi intercrops. *Plant Soil,* **108**, 125–135.

Sarkar, R. K., Pal, P. K. (2004): Effect of intercropping rice *(Oryza sativa)* with groundnut *(Arachis hypogaea)* and pigeonpea *(Cajanus cajan)* under different row orientation on rainfed uplands. *Indian J. Agron.*, **49,** 147–150.

Snapp, S. S. (1998): Phosphorus and sustainability of sub-Saharan African smallholder farms. pp. 71–83. In: Lynch, J. P., Deikman, J. (eds.), *Phosphorus in Plant Biology: Regulatory Roles in Molecular, Cellular, Organismic and Ecosystem Processes.* American Society of Plant Physiology, Rockville, MD.

Stinner, B. R., Blair, J. M. (1990): Ecological and agronomic characteristics of innovative cropping systems. pp. 123–140. In: Edwards, C. A., Lal, R., Madden, P., Miller, R. H., House, G. (eds.), *Sustainable Agricultural Systems*. St. Lucie Press, FL.

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ROOT DEVELOPMENT, SHOOT GROWTH AND YIELDS OF MAIZE AS AFFECTED BY IRRIGATION SCHEDULES IN A MINOR SEASON IN TROPICAL ASIA

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Maize is the most important upland cereal in tropical Asia, grown in both major and minor seasons under rainfed conditions. Due to the inadequate rainfall in the minor season, the crop is subjected to water stress, and irrigation helps to produce high yields. Smallholders who grow maize on flat beds in their allotments often use surface flood irrigation whenever irrigation water is available, which leads in most instances to inefficient use of this valuable resource. A field study was carried out over two minor seasons in Sri Lanka to determine the impact of different schedules of irrigation, developed on the basis of time intervals (3-, 7-, 14- or 21-day intervals or no irrigation as a control), which can easily be practised by smallholders, on the root development, shoot growth, seed yield and water use efficiency of maize. Irrigation at 3-day intervals produced fine roots in the top layers of the soil. Increasing the time interval between irrigation schedules to 7, 14 or 21 days reduced the percentage of fine roots, but developed more, heavier roots in the lower soil layers, as determined by root length densities (RLD) and root weight densities (RWD). Longer irrigation intervals or lack of irrigation resulted in a smaller number of heavier roots in the soil profile. The leaf water potential was affected to a greater degree than shoot water content or relative water content. The seed yield and harvest index were highest when maize was irrigated at 7-day intervals. In contrast, irrigation water use efficiency (IWUE) was highest at an irrigation interval of 14 days. The potential for optimizing water use in surface irrigation in flat beds while obtaining high yields in a tropical Asian minor season, when maize is subjected to moisture stress under smallholding conditions, is presented on the basis of this study.

Key words: maize, irrigation, root growth, shoots, water use efficiency, yield

Introduction

Sustainable agriculture in humid Asian tropics such as Sri Lanka is often limited by the lack of adequate water, especially in the minor season, when evaporation generally exceeds precipitation. Hence, irrigation is used in these circum-

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stances in most tropical regions with limited water resources in order to optimize crop production (Rockstrom, 2003). In most instances, smallholders cultivating crops in the minor season use flood irrigation, which leads to the wasteful use of this resource during times of scarcity. Hence, there is significant pressure to enhance the efficiency of water use in irrigation, through proper management strategies, while maintaining productivity (IWMI, 2000).

Water management strategies illustrate the benefits of irrigating crops at critical growth stages, although most smallholders have difficulty in adopting these practices under field conditions (Kirda et al., 2005). Hence, simple water-conserving irrigation practices are important for optimizing the use of available water and producing the highest yields under smallholder tropical farming conditions.

Many methods, both simple and sophisticated, are available for conserving irrigation water. Among these, wide-spaced furrows or skipped-row irrigation methods have increased water use efficiency significantly (Stone and Nofziger, 1993; Kang et al., 2000). These methods provide trade-offs between lower yields and greater water use efficiency. However, as most tropical smallholders use flood irrigation for flat beds for ease of management and low use of labour, these advanced methods cannot be used. Under such circumstances, conventional deficit irrigation methods could be easily adopted to reduce water use without significant losses in yields, although farmers need to know the critical growth stages of crops (Kirda et al., 2005). Hence, simple regulated irrigation frequencies would be more useful for farmer adoption, especially under flat bed field planting systems.

Maize (*Zea mays* L.) is the most popular highland cereal in South Asia for human consumption and animal feed, and is grown under rainfed conditions in major seasons and under either rainfed or irrigated conditions in the minor seasons. Yields of maize are comparatively lower in the minor seasons, especially under rainfed conditions, due to moisture stress, so if facilities are available, farmers irrigate their crops to enhance productivity, although water is fast becoming the most limiting abiotic factor for tropical crop yields (Turner, 2000). In the case of irrigated maize cultivation, optimal water management strategies become important, as irrigation water is limited in minor seasons and is also used on a priority basis for rice cultivation, due to its importance as the staple diet.

Divided root studies under controlled conditions and alternative furrow irrigation trials in the field (e.g. Kang et al., 2000; 2002), where the soil profile was watered both vertically and horizontally, highlight the possibility of stimulating the root growth of maize through alternative wetting and drying, thereby maintaining crop yields while reducing water use. However, the impact of such practices for flat bed maize cropping systems in terms of root dynamics, growth and yields of maize has not been reported. The objective of this study, carried out under field conditions in a minor season in Sri Lanka, was to assess the impact of different surface irrigation frequencies on root growth patterns, growth and yields of maize grown on flat beds, as per normal farm practice.

Materials and methods

The experiment was carried out at the Experimental Unit of the University of Peradeniya, Sri Lanka (8°N, 81°E, 418 m above sea level) in the mid-country intermediate zone of Sri Lanka, in the minor seasons of 2004 and 2005 (late April – August in both years), which corresponds to the south west monsoon. The soil of the site is an Ultisol (Rhododhult) with a pH (1:25 H_2O) of 6.96 ± 0.13, total N content of 56 mg g^{-1} soil, CEC of 38.5 ± 5.6 m. eq. 100 g^{-1} soil and organic C content of 0.68% ± 0.07 in the top 25 cm soil layer, which is considered to be the effective rooting zone.

The rainfall in these seasons in 2004 and 2005 was 412 mm and 398 mm, respectively, with mean air and soil temperatures of 31.34°C and 32.04°C, respectively, over the two seasons, without much interseasonal variation, with mean pan evaporation of 3.54 mm day^{-1} and 3.22 mm day^{-1} in the same time period. The mean relative humidity was 68.7% ± 2.84 over the seasons. Hence, crops grown in this season were subject to moisture deficit. The soil moisture content at field capacity was 0.21 g g^{-1}.

At the onset of the rains in late April of the two seasons, plots measuring 4 × 4 m were prepared manually as per farm practice. The border areas between plots were 75 cm wide to avoid the horizontal movement of irrigation water. Maize (open pollinated variety Bhadra – germination 94.5%) was planted at the recommended spacing of 60 × 30 cm to obtain a population of 55,000 plants ha^{-1}. The fertilizer applied was equivalent to 25 kg N, 45 kg P and 30 kg K per ha at planting, followed by 45 kg N at 45 days after planting. Manual weeding was adopted at two-week intervals to remove all weeds in order to overcome errors in root mass due to the presence of these plants.

The treatments imposed in the study were to irrigate predetermined plots at intervals of 3, 7, 14 or 21 days from planting up to 90 days, when the seeds began to ripen. However, one plot was maintained under rainfed conditions without any additional supply of water. The experiment, carried out on separate but adjacent plots to avoid carry-over effects, thus contained five irrigation treatments within a randomized block design, with four replicates. At each irrigation, water was provided manually to the respective plots at a rate of 100 litres per plot to bring the soil to field capacity. All possible care was taken to avoid the lateral movement of water between the different plots. The total volume of water added in the treatments was 3000, 1330, 600 and 400 litres per plot for irrigation regimes of 3-, 7-, 14- and 21-day intervals.

The measurements taken from all plots in both seasons were as follows:

At the V4 and V8 growth stages, the maize roots in the soil were sampled using core samples (5 cm diameter and 20 cm length). The soil of each plot was randomly sampled at 4 places, using a soil sampler at depths of 0–20, 20–40, 40–60 and 60–80 cm, as described by Böhm (1979). The soil cores were individually washed onto a 0.5 mm mesh and the roots were collected. The total root lengths of all samples were determined using the grid technique (Tennant, 1975) and the percentage of fine roots in a subsample from each depth based on visual observations of roots having an approximate diameter of 1 mm or less. The roots were then dried at 80°C before weighing. The root length density (RLD) and root weight density (RWD) were calculated as follows:

RLD (cm cm^{-3}) = Total length in a soil core/Volume of core
RWD (mg cm^{-3}) = Total dry weight of roots in core/Volume of core

At anthesis, the leaf area per plant was measured using a leaf area meter (Li Cor 4000, Li Cor, USA), the water potential of the topmost fully opened leaf in a Scholander pressure chamber (Scholander et al., 1965), and the shoot fresh and dry weights and root dry weights of 4 plants per plot after drying at 80°C for 48 hours. These values were used for calculating shoot water content and root:shoot ratios.

At crop maturity, the stover, root and seed yields were determined. The seed, root and stover were dried at 80°C for 48 hours prior to weight determination. The weights were used for calculating the root:shoot ratio, and the harvest index (HI) and relative crop yield (RCY) as follows:

Harvest Index (HI) = Seed yield/Stover yield
RCY = Yield of irrigated plots/Yield of rainfed plots (adapted from Garcia-Barrios, 2003).

The irrigation water use efficiency (IWUE) was calculated as follows:

IWUE = Seed yield (kg ha^{-1})/Volume of applied water (litres) (adapted from Kirda et al., 2005).

In all plots, three soil samples, taken to a depth of 80 cm using an auger, were used to determine soil moisture content prior to each irrigation. In the rainfed plots similar soil samples were taken at 21-day intervals. These were used to calculate the mean soil moisture content prior to each irrigation in the irrigated plots and the mean soil moisture content in the rainfed plots over the growing season.

As the response of the crops was similar in both seasons, the data were pooled before being subjected to appropriate statistical analysis using the GLM procedure for analysis of variance. Treatment differences were determined using probability ($P = 0.05$) or Fisher's protected LSD, and, when required, the data were transformed using logarithmic values to ensure normal distribution. The data presented are the means of two seasons.

Results and discussion

The mean soil moisture contents of soils just prior to irrigation every 3, 7, 14 or 21 days were 0.19, 0.16, 0.10 and 0.06 g g^{-1}, respectively, while the soil moisture content at field capacity was 0.21 g g^{-1}. The mean soil moisture content of non-irrigated plots was 0.5 g g^{-1}. This indicated that an irrigation frequency of 21 days could subject the crop to stress due to the low soil moisture throughout the growth period, which was similar to non-irrigated (i.e. rainfed) conditions. In contrast, irrigating at 3-day intervals maintained soil moisture contents near field capacity, while the mean depletion at a frequency of 7 days intervals was 23% of the moisture at field capacity. In contrast, the 14-day interval irrigation subjected the maize plants to drying and wetting cycles, as the mean soil moisture content was approximately 50% of the water at field capacity.

There were dynamic changes in the rooting patterns of maize due to the irrigation regimes adopted (Table 1). A reduced frequency of irrigation enhanced soil penetration by a greater volume of roots. The mean RLD at both samplings was increased by reducing the frequency of irrigation up to 14 days. However, the mean RLD declined when water was supplied at 21-day intervals or when maize was grown without irrigation in the minor seasons. This implied that overall root extension is stimulated by infrequent supplies of water up to intervals of 14 days, and maize plants produced fewer but heavier roots (as denoted by RWD) when soil moisture was limited, as observed with a 21-day irrigation frequency or under rainfed conditions. This phenomenon is also confirmed by the mean percentages of fine roots (Table 1) observed at both harvests. The most significant aspect was the lack of difference in the trends of all measured root parameters at the two harvesting dates, which implied that the response of maize roots to soil moisture and irrigation frequencies does not vary with time.

The provision of water at 3-day intervals produced a significantly greater volume of fine roots in the top 20 cm of the soil profile. The RWD was also high in this layer with this irrigation regime. However, RLD and RWD declined through

Table 1
Impact of irrigation regimes on mean root distribution patterns in the soil profile (pooled values of two seasons)

Irrigation schedule	Soil depth (cm)	RLD (cm cm^{-3})		RWD (mg cm^{-3})		% Fine roots[a]	
		V4	V8[b]	V4	V8	V4	V8
3-day interval	0–20	5.14	6.85	0.35	0.51	64	58
	20–40	3.68	4.15	0.16	0.31	61	54
	40– 60	2.04	3.26	0.11	0.26	58	51
	60 –80	1.15	2.05	0.06	0.14	56	51
	Mean	3.01	4.07	0.17	0.30	59.75	53.50
7-day interval	0–20	4.86	5.92	0.31	0.52	58	51
	20–40	4.14	4.95	0.25	0.39	55	50
	40–60	3.42	4.01	0.21	0.32	49	45
	60–80	2.25	2.96	0.14	0.25	46	43
	Mean	3.66	4.46	0.22	0.37	52	47.25
14-day interval	0–20	4.75	5.83	0.30	0.48	56	50
	20–40	4.56	5.12	0.28	0.42	54	48
	40–60	3.65	4.42	0.25	0.39	45	45
	60–80	2.84	3.15	0.21	0.32	41	44
	Mean	3.95	4.63	0.26	0.40	49	46.75
21-day interval	0–20	3.36	4.15	0.31	0.52	38	34
	20–40	3.18	4.64	0.36	0.56	35	33
	40–60	2.59	3.14	0.26	0.41	29	26
	60–80	2.31	2.68	0.22	0.38	34	29
	Mean	2.86	3.65	0.28	0.46	34	30.5
No irrigation	0–20	3.42	4.01	0.34	0.49	35	33
	20–40	3.02	4.12	0.33	0.51	34	31
	40–60	2.62	3.68	0.28	0.46	28	24
	60–80	2.38	3.32	0.26	0.42	31	27
	Mean	2.85	3.78	0.30	0.47	32	28.75
Probability ($P = 0.05$)	Irrigation	0.046	0.029	0.017	0.039	0.007	0.016
(n = 80)	Depth	0.022	0.043	0.031	0.029	0.018	0.032
Interaction		*	*	*	*	*	NS

[a] Fine and thick roots were separated on the basis of visual observation of washed root samples; [b] V4 and V8 refer to growth stages on the Feekes Scale; * significant at the 0.05 probability level; NS: non-significant; RLD and RWD: root length density and root weight density, respectively.

the soil profile, although the percentage of fine roots was highest at all depths in this treatment. This clearly implied that the presence of adequate soil moisture stimulates the formation of fine roots in the surface layers of soil. In contrast, longer intervals between irrigations stimulated root growth into deeper layers of soil. Both RLD and RWD increased significantly at both harvests in the deeper layers of soil when the maize plants were irrigated at 7- or 14-day intervals, with no change in trends between the two samplings. The RLD was highest for all soil profiles when the plants were irrigated at 14-day intervals. This phenomenon of root

stimulation by alternative wetting and drying could be attributed to the possibility of plant roots sensing soil drying by producing a root signal that regulates shoot growth to promote root development (Davies and Zhang, 1991; Kang et al., 2002). Detailed research has also shown that this root signal is due to the abscisic acid (ABA) concentration in the xylem (Jia et al., 1996). However, the percentage of fine roots developed with an irrigation interval of 14 days was significantly lower compared to that of plants with a 3-day interval, indicating root thickening with greater intervals between irrigations, a phenomenon not identified earlier.

An irrigation frequency of 21 days reduced RLD throughout the soil profile, while RWD increased. The percentage of fine roots also declined significantly, when compared to values at more frequent irrigations. This implied that maize plants produce fewer thicker roots that penetrate the soil profile to a greater depth to extract available soil moisture. This phenomenon is confirmed by the values obtained from plants grown without irrigation. These plants had the lowest RLD values throughout the soil profile. Furthermore, RWD was also higher in these plants, especially at the 40–80 cm depth. These results show that maize plants grown under rainfed conditions in the minor season, where the crop is subject to water stress, produce a few thicker (and possibly stronger) roots throughout the soil profile to extract the available moisture from deeper layers of the soil.

The highest leaf area per plant at anthesis was recorded in plants irrigated at 3- or 7-day intervals (Table 2) and the values declined with increasing intervals, with the lowest leaf area observed in plants grown without irrigation. This showed that the vegetative growth of plants grown under rainfed conditions in this minor season responds to irrigation. The leaf water potential of plants irrigated at 3-, 7- or 14-day intervals was not significantly different, illustrating that the newly matured leaves, which are the most active photosynthetically, had similar water contents. When plants were subjected to a 21-day schedule or grown only with rainfall, the shoot water potential increased significantly, indicating a lower leaf water content. The trend of changes in shoot water content was similar to that of leaf wa-

Table 2

Leaf area, leaf water potential and shoot water content of maize plants at anthesis as affected by irrigation scheduling (pooled values of two seasons)

Irrigation schedule	Leaf area (cm^2)	Leaf water potential (Mpa)	Shoot water content (%)	RWC* (%)
3 days	1946	–2.88	84.5	119
7 days	1855	–2.69	80.65	114
14 days	1493	–2.78	78.96	111
21 days	814	–3.82	74.64	105
None	727	–4.59	70.62	89.6
LSD ($P = 0.05$)		1.03	3.91	

* Relative water content (RWC) indicates the water content of irrigated plants in relation to that of non-irrigated plants

ter potential (Table 2), where plants irrigated at 3-day intervals had the highest values, with no significant differences in values recorded at irrigation intervals of 7 and 14 days. Again the lowest values were in plants not irrigated. Hence, the relative water contents of the plants were highest when maize was irrigated at 3- or

Table 3

Root dry weights and root:shoot ratios of maize at anthesis and harvest as affected by irrigation schedule (pooled values of two seasons)

Irrigation schedule	Anthesis		Harvest	
	Dry wt (g plant^{-1})	Root:shoot ratio	Dry wt (g plant^{-1})	Root:shoot ratio
3 days	165	0.14	241	0.17
7 days	184	0.29	319	0.31
14 days	250	0.37	384	0.38
21 days	206	0.30	239	0.25
None	186	0.26	249	0.24
LSD ($P = 0.05$)	14.6	0.05	43.8	0.07

7-day intervals, with a marginal reduction with an irrigation schedule of 14 days. In contrast, the shoot water contents of plants irrigated at 21-day internals were only marginally higher than in plants not irrigated. Regression analysis on changes in leaf water potential due to irrigation frequencies ($y = -0.1707x^2 + 0.5093x - 2.93$, $r^2 = 0.9686$) and shoot water contents ($y = -0.0025x^2 - 0.0325x + 1.2225$, $r^2 = 0.7905$) highlight the fact that leaf water potentials are more sensitive to soil moisture. The decline in leaf water potential was significantly greater with lower frequencies of irrigation, suggesting that leaves are more sensitive to soil moisture conditions than the entire shoot system, a phenomenon that requires further study.

The lowest root dry weights were recorded when the plants were irrigated frequently (3-day intervals). This signifies that although leaf area is greater when maize plants are irrigated at 3-day intervals, the plant roots had a lower dry matter content. The lower root:shoot ratio also signifies the development of a smaller root system in relation to the shoots, due to the presence of adequate soil moisture, which could affect the growth of plants if a dry period occurs at later growth stages. The highest root dry weights and thus the highest root:shoot ratio was observed in plants irrigated at 14-day intervals, which indicates the stimulation of roots due to wetting and drying of the soil at this frequency. This phenomenon was also evident in plants irrigated at a 7-day interval. An irrigation frequency of 21 days reduced the root dry weights at both samplings and thus the root:shoot ratios, which were similar to that of plants grown without irrigation. This suggests that maize grown with infrequent irrigation in the minor season is subjected to water stress to similar extents as plants grown without any irrigation.

Seed yields were highest when plants were grown at an irrigation frequency of 7 days and hence had the highest relative crop yields (RCY) compared to

Table 4
Effect of irrigation schedule on seed yield, harvest index and relative yield of maize (pooled values of two seasons)

Irrigation schedule	Seed yield (kg ha^{-1})	IWUE (kg seed/l water)	HI	RCY*
3 days	2495	0.83	0.42	1.63
7 days	2528	1.94	0.40	1.65
14 days	2348	3.32	0.39	1.56
21 days	1726	4.31	0.35	1.12
None	1528	–	0.31	–
LSD ($P = 0.05$)	418.7	1.06	0.004	

IWUE = Irrigation water use efficiency; HI = Harvest Index; RCY = Yield with irrigation/Yield with no irrigation

non-irrigated plants (Table 4). This suggests the benefits of irrigating maize in this minor season at 7-day intervals to obtain maximum seed yields rather than at higher or lower frequencies. The seed yields of maize irrigated at 14-day intervals were lower (7%) than that of plants irrigated every 7 days, but this difference was not statistically significant. Furthermore, the RCY and harvest indices of plants irrigated every 3, 7 or 14 days were similar. However, as expected, the lowest seed yields and harvest indices were observed when the plants were irrigated every 21 days or grown without irrigation. This clearly indicated the requirement for irrigation to produce high yields when maize was grown conventionally in the minor season in Sri Lanka, which is similar to most tropical Asian humid regions. However, the supply of irrigation water even at a frequency of 21 days over the first 90 days of this 110-day crop increased seed yields by 12%, which substantiates the above statement.

Irrigation water is fast becoming a scarce commodity and hence available water needs to be used efficiently (Turner, 2000), as excessive supplies could lead to the development of salinity. The highest irrigation water use efficiency (IWUE) in this study, calculated on a unit volume basis, was recorded for an irrigation schedule of 14 or 21 days (Table 4). The supply of water every 3 days produced the lowest IWUE, clearly indicating the very low use efficiency of this valuable resource. Furthermore, such frequent irrigation could help leach mobile nutrients such as nitrogen, which is very limiting in tropical soils, and also cause salinity development. When irrigation was provided at 7-day intervals IWUE was higher than at 3-day intervals, but significantly lower than that recorded at a 14-day interval. However, the seed yield obtained at an irrigation interval of 14 days was 36% greater than that obtained at a frequency of 21 days, which was a significant increase. Hence, the irrigation schedule to produce the optimal seed yield with a very high IWUE would be a frequency between 7 and 14 days.

Conclusions

A well-developed root system is vital for absorbing soil moisture. This becomes especially important in minor dry seasons in the tropics, where crops are often subject to moisture stress. Research under controlled conditions (e.g. Kang et al., 1998) and under alternative furrow irrigation systems in the field (Kang et al., 2000; 2002) highlight the stimulation of maize roots under alternative wetting and drying. This phenomenon was confirmed in the present study over two minor seasons, when the phenomenon was investigated under uniform surface irrigation, which is akin to the flood irrigation commonly practised by smallholders in the tropics. This stimulation of root growth was manifested in terms of both RLD and RWD throughout the soil profile when water was supplied at intervals of 7 or 14 days. This in turn maintains a high leaf water potential and shoot water content to avoid moisture stress. The greater root dry weights at anthesis and harvest also signify this phenomenon of stimulation to provide a better root system, which is supported by the higher root:shoot ratios.

Farmers need to obtain high crop yields with limited use of scarce resources such as water. Although the highest yield was obtained at an irrigation interval of 7 days, the yield reduction with an irrigation schedule of 14 days was marginal when compared to the significant increase in IWUE. This clearly implied the benefits of supplying irrigation water at an interval between 7 and 14 days to optimize yields, to avoid the possible leaching of nutrients such as nitrogen and to obtain the highest water use efficiency. Confirmatory studies are in progress to monitor the overall benefits of providing irrigation water to optimize nitrogen nutrition, obtain high yields and procure the maximum utility value from the supplied water.

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References

Böhm, W. (1979): *Methods of Studying Root Systems*. Springer-Verlag, Berlin, Germany.

Davies, W. J., Zhang, J. (1991): Root signals and the regulation of growth and development of plants in drying soil. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **42**, 55–76.

Garcia-Barrios, L. (2003): Plant-plant interactions in tropical agriculture. pp. 11–58. In: Vandermeer, J. H. (ed.), *Tropical Ecosystems*. CRC Press, Washington, USA.

IWMI (2000): *Water Supply and Demand in 2025*. International Water Management Institute, Colombo, Sri Lanka.

Jia, W., Zhang, J., Zhang, D. P. (1996): Metabolism of xylem delivered ABA in relation to ABA flux and concentration in leaves of maize and *Commelina cummunis*. *J. Expt. Bot.*, **47**, 1085–1091.

Kang, S., Liang, Z., Hu, W., Zhang, J. (1998): Water use efficiency of controlled alternate irrigation on root-divided maize plants. *Agric. Water Manage.*, **38**, 69–76.

Kang, S., Shi, W., Cao, H., Zhang, J. (2002): Alternate watering in soil vertical profile improved water use efficiency of maize (*Zea mays*). *Field Crops Res.*, **77**, 31–41.

Kang, S., Liang, Z., Pan, Y., Shi, P., Zhang, J. (2000): Alternate furrow irrigation for maize production in arid area. *Agric. Water. Manage.*, **45**, 267–274.

Kirda, C., Topcu, S., Kaman, H., Ulger, A. C., Yazici, A., Cetin, M., Derici, M. R. (2005): Grain yield response and N fertilizer recovery of maize under deficit irrigation. *Field Crops Res.*, **93**, 132–141.

Rockstrom, J. (2003): Water for food and nature in drought prone tropics: vapor shift in rainfed agriculture. *Phil. Trans. R. Soc. Lond.*, **358**, 1997–2009.

Scholander, H. T., Mahel, E. D., Bradstreet, E. A., Hemmingsen, S. S. (1965): Sap pressure in vascular plants. *Science,* **148**, 339–346.

Stone, J. F., Nofziger, D. L. (1993): Water use and yields of cotton grown under wide spaced furrow irrigation. *Agric. Water. Manage.*, **24**, 27–43.

Tennant, D. (1975): A test of a modified line intercept method of estimating root length. *J. Ecol.*, **63**, 993–1001.

Turner, N. (2000): Optimizing water use. pp. 119–137. In: Noesberger, J., Geiger, H. H., Stuik, P. P. (eds.), *Crop Science – Prospects and Progress*. CABI, Wallingford, U.K.

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EFFECT OF YEAR AND TECHNOLOGICAL FACTORS ON THE YIELD AND AGRONOMIC TRAITS OF SWEET CORN (*Zea mays* L. CONVAR. *saccharata* KOERN.) VARIETIES IN A LONG-TERM EXPERIMENT

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The effect of three technological factors (sowing date, fertilization, crop density) and two genotypes was examined on the yield and agronomic traits of sweet corn grown on chernozem soil in the Hajdúság region of Hungary. The experiments, conducted at the Látókép Research Site of the University of Debrecen, involved two sowing dates (end of April, end of May), six fertilization levels (control, N_{30} + PK, N_{60} + PK, N_{90} + PK, N_{120} + PK, N_{150} + PK) and two plant density levels (45 thousand ha^{-1}, 65 thousand ha^{-1}). Both hybrids used (Jumbo and Enterprise) belong to the mid-late maturity group. Compared to the 30-year average, the climate was dry and warm in 2009. Moisture deficiency had an adverse effect on the yield of crops sown at the second sowing date. By contrast, the second year (2010) was very humid; the precipitation was 184 mm above the 30-year average and the temperature was average.

In the dry year the highest yield was obtained with Jumbo for both sowing dates (27253 kg^{-1}, 20921 kg ha^{-1}), while in 2010 Enterprise gave the highest yield (23437 kg^{-1}, 22237 kg ha^{-1}) for both sowing dates. The correlation between the technological factors, the yield and agronomic traits was analysed using Pearson's correlation coefficient.

Key words: sweet corn, plant density, sowing date, fertilization, yield, cob number

Introduction

Sweet corn is a subspecies of maize; it differs from maize in that it has a recessive gene (sugary endosperm) on the fourth chromosome, which allows only partial transformation of the accumulated sugars into starch (Balázs, 1944). Thanks to its sweet taste, sweet corn is a popular food; it is preserved by canning or freezing (Oktem et al., 2003). The total production area of sweet corn on a global scale is around 500 thousand ha. The leading sweet corn producers are the USA and Canada, but Hungary is also important, with exports of over 100 thou-

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sand tonnes (Zsombik and Daróczi, 2008). As regards the marketing of sweet corn products, it is worth emphasizing the GMO-free status of Hungary, which could gain importance in the future (Kovács, 2005).

It has the longest sowing period of all the field vegetables, covering three months from mid-April (early sowing) to early July (late sowing) (Williams, 2008). Based on experiments carried out in 2005 and 2006, Kumar (2009) found that the optimal plant density for sweet corn is 83 thousand plants ha^{-1} and the optimal fertilization level 120 kg ha^{-1} N. According to the calculations of Debreceni (2005), the dry matter production of sweet corn may be as much as 245 kg ha^{-1} day^{-1}. This rate decreases to 204, 200 and 82 kg ha^{-1} day^{-1} in the case of phosphorus, potassium and nitrogen deficiency, respectively. Sárvári (2000) showed that there may be differences of over 50% between the nutrient use efficiency values of individual sweet corn hybrids. According to Hodossi et al. (2004), the optimum crop density ranges between 40 thousand and 70 thousand plants ha^{-1}. Hallauer and Miranda (1981) calculated the heritability (h^2) of the yield components of sweet corn hybrids and found that the number of kernel rows was least affected by environmental factors. Veneni (1971) examined the variability of yield components and found that the variability of kernel weight per cob was highest for crop years and varieties (CV = 16.8–35.4%), followed by cob length (CV = 9.2–15.6%) and cob diameter (CV = 4.66–8.24%). Analysing the stability of yield components, Gyenesné Hegyi et al. (2002) found that individual cob production (CV = 79.3–42.4%) and kernel weight (CV = 17.5–30.0%) were the most variable traits. The variability of the main cob was medium (CV = 77.1–13.3%), while the most stable yield component, least affected by environmental effects, was the kernel number per row (CV = 76.6–9.2%).

Material and methods

The experiment was carried out at the Látókép Plant Research Site of Debrecen University, located on a loess ridge in the Hajdúság region. Based on its physical characteristics, the soil was semi-compacted clay with good physical status.

The precrop of the examined stands was winter wheat, which is an excellent precrop for sweet corn.

In 2009 two commercially produced hybrids in the mid-late maturity group, Jumbo and Enterprise, were sown at two sowing dates: early, at the beginning of the main sowing period (April 21, 2009 and April 27, 2010) and late, at the end of the main sowing period (May 19, 2009 and 26 May, 2010).

The experiment involved six fertilization levels (control, N = 30 kg ha^{-1}, P_2O_5 = 22.5 kg ha^{-1}, K_2O = 26.5 kg ha^{-1} as base fertilizer, and 2, 3, 4 and 5 times these quantities) and two plant density levels (45 thousand ha^{-1}, 65 thousand ha^{-1}) in both years.

The plots were laid out in a random block design with four replications; the plot size was 11.4 m^2. The sweet corn cobs were harvested by hand in the husk. The moisture content of the kernels ranged between 67 and 69%, which is optimal for the canning industry.

Table 1 shows the monthly precipitation and temperature values in the examined years.

In 2009 the total amount of precipitation in the vegetation period for the early sowing date was 110.6 mm below the 30-year average and the average temperature was 2.5°C above the 30-year

Table 1
Meteorological data for the growing periods (Debrecen)

Month	Monthly precipitation (mm)			Monthly average temperature (°C)		
	2009	2010	30-year average	2009	2010	30-year average
April	9.9	83.3	42.4	14.9	11.6	10.7
May	20.1	111.4	58.8	17.4	16.6	15.8
June	96.6	100.9	79.5	19.8	19.7	18.7
July	9.2	97.2	65.7	23.4	22	20.3
August	11.3	98.3	60.7	22.6	19	19.6
Total/average	147.1	491.1	307.1	19.6	17.8	17

average (April, May, June, July). For the late sowing date the moisture deficiency was even higher (160 mm), while the temperature was 2.6°C above the average (April, May, June, July, August). By contrast, the 2010 growing season was outstandingly wet. For the early sowing date, the total amount of precipitation in the vegetation period was 339 mm, which was 93 mm above the 30-year average; the average temperature was 1.5°C above the 30-year average (April, May, June, July). For the late sowing date the surplus precipitation was 24.7 mm and the temperature was 0.7°C above the 30-year average.

Results

Hybrids have different yield potential, which is genetically determined. Yield potential is equally influenced by the year and the technology.

In 2009 the total precipitation in the 5-month growing season was 160 mm below the 30-year average. The greatest moisture deficiency was detected in July, when the rainfall was only 14% (9.2 mm) of the average amount (65.7 mm). June was the only month when the precipitation was 17.1 mm above the 30-year average (96.6 mm). In the case of the early sowing date, the moisture deficiency in April and May was well compensated by the moisture accumulated in autumn and winter, while the precipitation in June provided sufficient moisture until the end of the vegetation period. By contrast, due to the very low precipitation values in July (9.2 mm) and August (11.3 mm) there was significant yield loss for the second sowing date.

In 2010, the precipitation exceeded the 30-year average by 184 mm in the vegetation period (from April to August). In the first month the precipitation was twice as high and the temperature was almost 1°C higher than the 30-year average for the first sowing date (April). Similarly, in May (second sowing date) the precipitation was almost double and the temperature was 0.8°C above the 30-year average. The excessive precipitation and the extreme temperature values caused the yellowing of the leaves. For the first sowing date the temperature in the flowering stage (beginning of July) exceeded the 30-year average by 1.7%, while the precipitation was almost 1.5 times higher. Only in August was the monthly mean temperature below the average, but even in this month the precipitation was 31.5 mm

higher than the average. Due to the excessive precipitation the vertical movement of soil nutrients increased and unfavourably influenced plant nutrient uptake. In addition, the soil compaction caused by intensive rainfall caused anaerobic soil conditions.

Technological factors (sowing date, crop density, fertilization) and two sweet corn genotypes were investigated in small-plot field experiments under excellent conditions at the Látókép Research Site of the University of Debrecen.

Sweet corn has a high nutrient requirement and makes good use of both the natural nutrient supplies of the soil and artificial fertilizers. The efficiency of fertilization is significantly influenced by soil conditions, water supply, genotypes and technological factors.

An analysis of the cob yield (Tables 2 and 3) revealed that the number of cobs was higher for the first sowing date in both years. In the dry year of 2009 this was due to the fact that the warm weather was favourable for sweet corn production. Due to the higher plant density level, both hybrids gave the highest cob yield at 65 thousand ha^{-1} at the N_{120}+PK fertilization level. Due to the very dry conditions in the second sowing date treatment the cob yield of both hybrids was highest on the control plots. In the extremely wet year of 2010 the cob yield was highest for the first sowing date. The less favourable weather after the second sowing date caused the cob yield per plant to decrease. In 2010, higher cob yields were obtained at the higher plant density. In the N_{120}+PK treatment, the cob yields of Jumbo and Enterprise ranged from 52,302–71,710 cobs ha^{-1} and 43,158–72,368 cobs ha^{-1}, respectively. Both at the control and the N_{120}+PK fertilization levels Jumbo gave the highest cob yields in the dry year and Enterprise in the humid year when sown early (Fig. 1). The highest cob yield was harvested at the 65 thousand ha^{-1} crop density level for the first sowing date in 2009 and for both sowing dates in 2010, while the highest cob yield was harvested at the 45 thousand ha^{-1} crop density level for the second sowing date in 2009. It was thus concluded that the water supply had a great influence on the cob yield.

The optimal fertilization levels for the individual hybrids were determined by applying various fertilizer doses. In Tables 4 and 5 numbers indicate the agroecological maximum yield of the hybrid. The agroecological fertilizer optimum is the level above which the increase in yield is not significant. In 2009, the yields obtained with the agroecological fertilizer optimum were similar for Jumbo and Enterprise in the early sowing date treatment at both plant density levels (Table 4). The agroecological optimum was recorded for Jumbo at N_{90}+PK (25,674 kg ha^{-1}) at the lower plant density level and at N_{120}+PK (27,253 kg ha^{-1}) at the higher crop density level, while for Enterprise the agroecological optimum was obtained at N_{60}+PK (25,592 kg ha^{-1}) and N_{120}+PK (26,382 kg ha^{-1}). When sown late, the yields of Enterprise were higher at both plant density levels (45 thousand ha^{-1}: 22,270 kg ha^{-1}, 65 thousand ha^{-1}: 21,513 kg ha^{-1}) and the highest yields of both hybrids were obtained at the control fertilization level. Increasing the fertilizer dose resulted in yield depression due to water deficiency.

Table 2
Effect of technological factors on the cob yield number ha–1 (Debrecen, 2009)

Sowing date	Plant density	Hybrid (A)/Fert(B)	Ø	N_{30}+PK	N_{60}+PK	N_{90}+PK	N_{120}+PK	N_{150}+PK	$LSD_{5\%}$	
Early	45 thousand ha^{-1}	Jumbo	61842	72368	68421	71052	77631	68421	10874.8 (A)	9724
		Enterprise	59210	67155	67105	69736	67305	61842	6876.2 (B)	(A×B)
	65 thousand ha^{-1}	Jumbo	72368	77631	80263	77631	84210	73684	7116.2 (A)	8274
		Enterprise	64473	67105	67105	71052	72368	64473	5850.4 (B)	(A×B)
Late	45 thousand ha^{-1}	Jumbo	47368	447937	44737	48684	36842	46052	10114.9 (A)	9852
		Enterprise	57894	48684	51315	46152	44939	47368	6966.0 (B)	(A×B)
	65 thousand ha^{-1}	Jumbo	49460	42105	46082	46052	44737	38158	8416.3 (A)	7805
		Enterprise	50000	46042	43421	44797	46052	35526	5518.7 (B)	(A×B)

Table 3
Effect of technological factors on the cob yield ha–1 (Debrecen, 2010)

Sowing date	Plant density	Hybrid (A)/Fert(B)	Ø	N_{30}+PK	N_{60}+PK	N_{90}+PK	N_{120}+PK	N_{150}+PK	$LSD_{5\%}$	
Early	45 thousand ha^{-1}	Jumbo	45789	48684	51873	51773	55911	60921	6763.1 (A)	7697
		Enterprise	46052	53947	56579	69079	67105	65789	3855.2 (B)	(A×B)
	65 thousand ha^{-1}	Jumbo	49737	55921	64868	67105	71710	68915	6013.1 (A)	5303
		Enterprise	64473	63157	63815	71910	72368	71710	2644.7 (B)	(A×B)
Late	45 thousand ha^{-1}	Jumbo	43092	41118	47910	46710	52302	42434	7763.1 (A)	6697
		Enterprise	39605	40131	40789	39802	43158	41118	3355.2 (B)	(A×B)
	65 thousand ha^{-1}	Jumbo	52631	52604	53289	50658	57894	53618	6907.8 (A)	7868
		Enterprise	49342	51644	53947	52678	53947	52237	3934.2 (B)	(A×B)

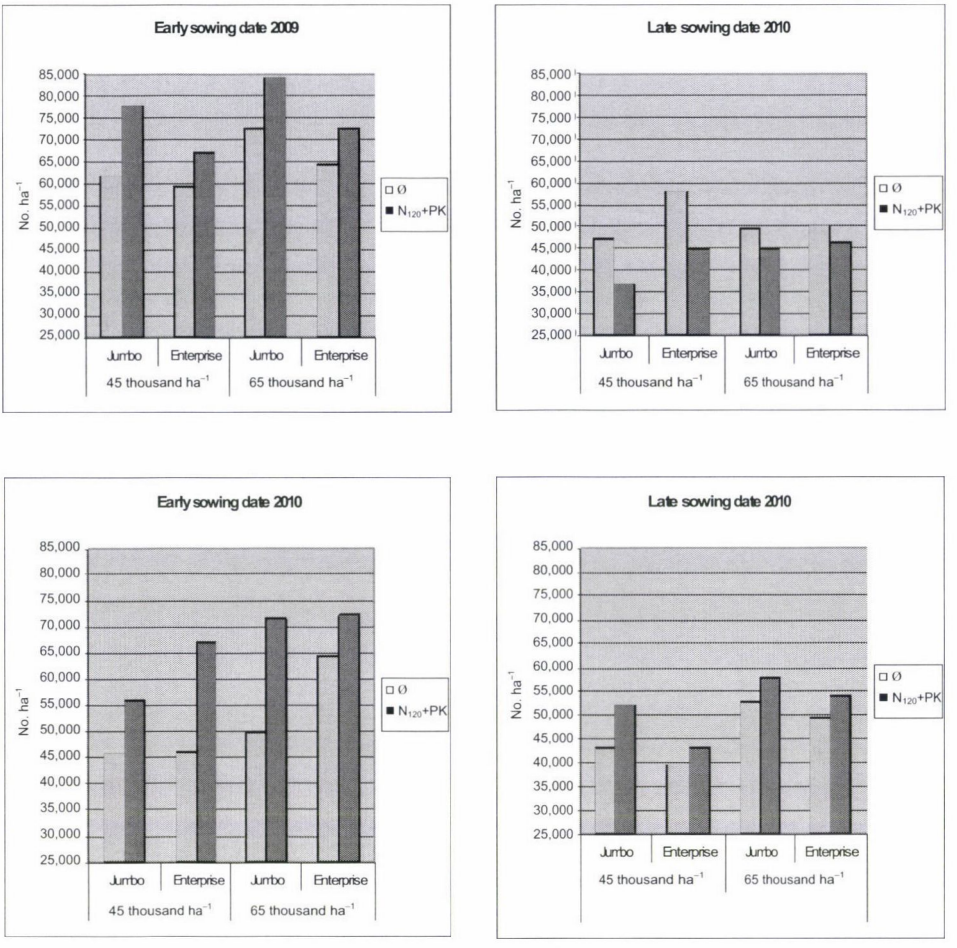

Fig. 1. Cob yield of the sweet corn hybrids Jumbo and Enterprise at the control and N_{120}+PK fertilization levels (number ha^{-1})

In 2010 yields (Table 5) Enterprise gave higher yields for both sowing dates (early sowing: 23,061 kg ha^{-1}, late sowing: 22,237 kg ha^{-1}) at the agroecological optimum fertilization level (N_{150}+PK). When sown late there was a significant difference between the average yields obtained with the agroecological fertilization optimum for Jumbo and Enterprise at the higher plant density level.

The effect of fertilizer treatments on the increase in yield was examined based on the control treatment, representing the natural nutrient supplies of the soil, which were utilized to different extents in the different years. This gives a good picture of the effect of individual fertilizer levels on the increase or decrease in yield, which can be considered as abiotic stress (Bocz, 1976). In the dry year (2009) the yield on control plots was relatively high (22,336–24,589 kg ha^{-1}) for the early sowing date, indicating the sufficient uptake and utilization of soil nutri-

Table 4

Effect of technological factors on the yield of sweet corn hybrids (kg ha^{-1}) (Debrecen, 2009)

Sowing date	Plant density	Hybrid (A)/Fert(B)	Ø	N_{30}+PK	N_{60}+PK	N_{90}+PK	N_{120}+PK	N_{150}+PK	$LSD_{5\%}$	
Early	45 thousand ha^{-1}	Jumbo	22401	22270	24967	**25674**	27007	24243	3322 (A)	
		Enterprise	22336	24605	**25592**	25312	25132	22220	2029 (B)	2964 (A×B)
	65 thousand ha^{-1}	Jumbo	24589	25806	26464	25757	**27253**	24194	2143 (A)	
		Enterprise	22385	24260	24095	25526	**26382**	24013	1762 (B)	2491 (A×B)
Late	45 thousand ha^{-1}	Jumbo	17187	17747	17286	**19145**	14507	17944	4130 (A)	
		Enterprise	**22270**	17862	19161	17368	15757	17681	2160 (B)	3054 (A×B)
	65 thousand ha^{-1}	Jumbo	**20921**	17812	19572	19293	19194	17072	2925 (A)	
		Enterprise	**21513**	20444	18569	19441	19128	14852	2405 (B)	3401 (A×B)

Table 5

Effect of technolgical factors on the yield of sweet corn hybrids (kg ha^{-1}) (Debrecen, 2010)

Sowing date	Plant density	Hybrid (A)/Fert(B)	Ø	N_{30}+PK	N_{60}+PK	N_{90}+PK	N_{120}+PK	N_{150}+PK	$LSD_{5\%}$	
Early	45 thousand ha^{-1}	Jumbo	12582	15395	15937	16809	17007	**18487**	3991 (A)	
		Enterprise	16612	19457	20428	22500	22105	**23437**	985 (B)	1970 (A×B)
	65 thousand ha^{-1}	Jumbo	14296	15543	19030	20099	**22253**	21464	2000 (A)	
		Enterprise	16546	18536	20641	20444	**23061**	22204	1024 (B)	2048 (A×B)
Late	45 thousand ha^{-1}	Jumbo	13470	15724	17056	17730	**18454**	16414	2727 (A)	
		Enterprise	16266	17714	17993	18536	18273	**18635**	1134 (B)	2267 (A×B)
	65 thousand ha^{-1}	Jumbo	18289	18882	18980	17845	**20888**	18602	2132 (A)	
		Enterprise	18306	19687	21546	20444	21546	**22237**	1214 (B)	2428(A×B)

ents. In this case increasing fertilizer levels caused no significant increase in the yield (10.8–17.9% surplus yield). For the late sowing date, the yields decreased at all fertilization levels. By contrast, in the humid year the yield on the control plots was well below that obtained at the agroecological fertilizer optimum. This could be due to the intensive precipitation, which leached the nutrients out of the root zone, or to immature roots or excessive vegetative mass. The yield increase was significantly higher in 2010 than in the dry year, ranging from 39.4 to 55.7% for the early sowing date and 14.2 to 37.0% for the late sowing date.

Pearson's correlation coefficient was calculated to analyse the correlation between the technological factors, yields and the agronomic traits of the harvested cobs in the two years (Table 6). In 2009 a weak negative correlation was found (–0.067) between fertilization and yield. This could be attributed to soil moisture

Table 6
Pearson's correlation coefficient for the factors examined in two years (Debrecen)

	2009	2010
Fertilizer × yield	–0.067	0.528**
Sowing date × cob weight (with husk)	0.502**	0.484*
Crop density × cob diameter	–0.711**	–0.178*
Cob weight (with husk) × kernel number	0.531**	0.519**
Cob weight × kernel number	0.332**	0.556**
Cob weight (with husk) × cob weight	0.590**	0.871**
Cob length × kernels per row	0.164*	0.648**

*, **: Differences significant at the P = 0.05 and P = 0.01 levels, respectively

deficiency, which reduced the efficiency of fertilization. In contrast, a medium positive correlation was found between these factors in 2010. In both years a medium positive correlation was found between sowing date and cob weight (0.502–0.484). In both the dry and the humid year the highest cob weight was obtained for the first sowing date. A strong negative correlation (–0.711) was found between crop density and cob diameter in 2009; the optimal plant density was lower due to the dry weather. Strong and very strong correlations (0.590–0.871) were found between cob weight (with or without husks) in both 2009 and 2010.

Conclusions

The early sowing date was found to be more favourable for sweet corn production in 2009, when the soil moisture content was sufficient for the plants. The number of healthy harvested cobs was highest in this treatment and the yield of both hybrids was above 24 t ha^{-1}. In the lower crop density treatment the agroecological fertilizer optimum was lower (N_{60}+PK for Enterprise, N_{90}+PK for Jumbo) than for the higher crop density (N_{120}+PK). The late sowing date aggravated the unfavourable effects of dry, hot weather. Averaged over the fertilizer

levels, the yields of both Jumbo and Enterprise exceeded 19 t ha^{-1}, while the highest yields were obtained with both hybrids on the control plots. In both treatments the higher plant density level proved to be better.

In 2010, no significant differences were found (16–20.5 t ha^{-1}) between the average yields of the two hybrids for the two sowing dates. However, the maximum yields were obtained for the first sowing date (18,487 kg ha^{-1} and 23,437 kg ha^{-1}). At the same time, the difference between the plant density levels was mitigated by the sufficient water supplies provided by the first, more favourable sowing date; at the low crop density the plants were able to compensate for the smaller number of plants by producing a second cob. Nevertheless, the interval of the agroecological fertilizer optimum was found to be wider (N_{60-120}+PK) for the early sowing date than for the second sowing date ($N_{120-150}$+PK), irrespective of the treatments and hybrids.

References

Balázs, S. (1994): *Zöldségtermesztők kézikönyve.* (Handbook of Horticulture.) Mezőgazda Kiadó, Budapest, pp. 631–639.

Bocz, E. (1976): *Trágyázási útmutató.* (Fertilization Guidelines.) Mezőgazdasági Kiadó, Budapest, pp. 117–146.

Debreczeni, B. (2005): Korszakváltás a tápanyag-gazdálkodásban. (Era change in nutrient management.) pp. 66–70. In: Pepó, P. (ed.), *Korszakváltás a hazai mezőgazdaságban: a modern növénytermesztés alapjai.* Debreceni Egyetem Agrártudományi Centrum.

Gyenesné Hegyi, Z., Pók, I., Zsubori, Z., Kizmus, L., Nagy, E., Marton, L. C. (2002): A kukorica (*Zea mays* L.) növénymagasságának és főcső eredési magasságának alakulása eltérő termőhelyeken. (Development of plant height and cob attachment height of maize at different growing sites.) *VIII. Növénynemesítési tudományos napok.* Budapest. p. 87.

Hallauer, A. R., Miranda, J. B. (1981): Hereditary variance: Experimental estimates. pp. 115–158. In: *Quantitative Genetics in Maize Breeding.* The Iowa State University, Ames, IA.

Hodossi, S., Kovács, A., Terbe, I. (2004): *Zöldségtermesztés szántóföldön.* (Field Horticulture.) Mezőgazda Kiadó, Budapest, pp. 340–348.

Kovács, F. (2005): Csemegekukorica leíró fajtakísérletek az OMMI-nál. (Descriptive variety trials on sweet corn hybrids at OMMI.) *Gyakorlati Agrofórum,* **16**(4), 22–25.

Kumar, A. (2009): Production potential and nitrogen-use efficiency of sweet corn *(Zea mays)* as influenced by different planting densities and nitrogen levels. *Indian J. Agric. Sci.,* **79**, 351–355.

Oktem, A., Simsek, M., Oktem, A. (2003): Deficit irrigation effects on sweet corn (*Zea mays* convar. *saccharata* Sturt) with drip irrigation system in a semi-arid region. I. Water yield relationship. *Agricultural Water Management,* **61**, 63–74.

Sárvári, M. (2000): Fajtaspecifikus kukoricatermesztési technológiák fejlesztése. (Development of hybrid-specific technologies for maize production.) *Agrofórum,* **11**(3), 53–55.

Veneni, M. (1971): Results of the study of maize hybrids for grain production under irrigated conditions. *Vedecke Prace Vyskumneho Ustavu Zavlahoveho Hospodarstva v Bratislave,* **9**, 147–178.

Williams, M. (2008): Sweet corn growth and yield responses to planting dates of the north central United States. *Hortscience,* **43**, 1775–1779.

Zsombik, L., Daróczi, M. (2008): A csemegekukorica termesztésének helyzete és aktualitásai. (Current situation of sweet corn production.) *Agrárágazat,* **9**(2), 24–26.

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LOW TEMPERATURE AND OXIDATIVE STRESS IN CEREALS

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Low temperature stress results in significant yield losses in cereals. Cereals of subtropical origin like maize and rice are severely damaged at temperatures below 10°C and are killed at subzero temperatures. This stress effect is called chilling. In contrast, cereals originating from the temperate zone (wheat, barley, rye and oat) may survive short periods even between –10 and –20°C, depending on the species and varieties, so they are freezing-tolerant to various extents. For the winter type of these cereals a gradual decrease in temperature up to –4°C results in cold acclimation, which increases their freezing tolerance. In addition, it fulfils their vernalization requirement, which is necessary for the correct timing of the vegetative to generative transition. During both chilling and freezing, oxidative stress is induced. Although the accumulation of high concentrations of reactive oxygen species may be lethal, a moderate increase in their level may activate various defence mechanisms. In this review the role of reactive oxygen species, antioxidants, carbohydrates, free amino acids, polyamines and hormones in the response to low temperature stress in cereals will be described. The effect of light and the use of the model plant *Brachypodium distachyon* L. to reveal the biochemical and molecular biological background of this response will also be discussed.

Key words: antioxidants, chilling, cereals, cold acclimation, freezing, reactive oxygen species, photoinhibition, redox signalling

Introduction

The exposure of plants to unfavourable environmental conditions is usually accompanied by the production of reactive oxygen species (ROS; Fig. 1), such as superoxide radicals, hydrogen peroxide, hydroxyl radicals and singlet oxygen (Okuda et al., 1991; Foyer et al., 1994a,b; Alscher et al., 1997; Suzuki and Mittler, 2006). The excessive accumulation of ROS may induce oxidative damage to pro-

Singlet oxygen	$^1O_2$
Superoxide anion	$\bullet O_2^-$
Perhydroxyl radical	HOO•
Hydrogen peroxide	H_2O_2
Hydroxyl radical	•OH
Hydroxide ion	OH^-

Fig. 1. Nomenclature of reactive oxygen species

teins, DNA and membrane lipids, or even cell death (Kendall and McKersie, 1989; Apel and Hirt, 2004). The ability to adjust the antioxidant system to changing ROS concentrations is vital in all species especially under stress conditions. In order to keep the amount of ROS in equilibrium, plants have evolved several enzymatic and non-enzymatic antioxidant systems. Thus, excess H_2O_2 can be removed by catalase (EC 1.11.1.6), guaiacol peroxidase (POD, EC 1.11.1.7) and the ascorbate-glutathione cycle (AsA-GSH cycle) (Foyer and Halliwell, 1976; Cakmak and Marschner, 1992; Prasad et al., 1994a; O'Kane et al., 1996; Kocsy et al., 2001b; Horváth et al., 2007). Ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR, EC 1.6.5.4) are the key enzymes in the AsA-GSH cycle in chloroplasts, which may also provide photoprotection (Noctor and Foyer, 1998; Mittler, 2002; Pang et al., 2005). However, ROS not only cause oxidative damage to cells, but may also play an important role as signals in plants exposed to various stress conditions (Mittler, 2002; Gupta and Luan, 2003; Mittler et al., 2004). Besides changes in ROS concentration, alterations in the ratio of GSH to glutathione disulphide (GSSG) and ascorbate to dehydroascorbate are important in stress signalling (Foyer et al., 1997). The interaction between ROS and antioxidants may provide the metabolic contact point between signals originating from metabolic pathways and the environment, thus regulating the induction of adaptive or death processes (Foyer and Noctor, 2005) through the activation of various proteins, specific transcription factors and antioxidants (Aslund and Beckwith, 1999; Polidoros and Scandalios, 1999; Vranová et al., 2002).

Low temperature is one of the most important stress factors limiting the growth and productivity of cereals. Chilling injury may occur when plants are exposed to low but not freezing temperatures for enough time to impair their life processes. During this period the tissues, unable to carry on normal metabolism, gradually become weakened. Chilling injury is therefore relatively slow. Freezing injury results when ice crystals form in the tissues, leading to severe osmotic and mechanical stresses. It usually occurs if plants are subjected to temperatures lower than the freezing points of their tissues, and it may take place in a few hours or even less, depending on the temperature. Ice generally forms first in the extracellular space; water then moves out of the cell along the osmotic gradient thus created, and osmotic stress is thereby imposed. Mechanical damage includes expansion-induced lysis, phase transitions and fracture lesions in membranes, and physical damage can be caused simply by the formation of large ice crystals. In addition, freezing may induce the production of ROS, which damage membrane

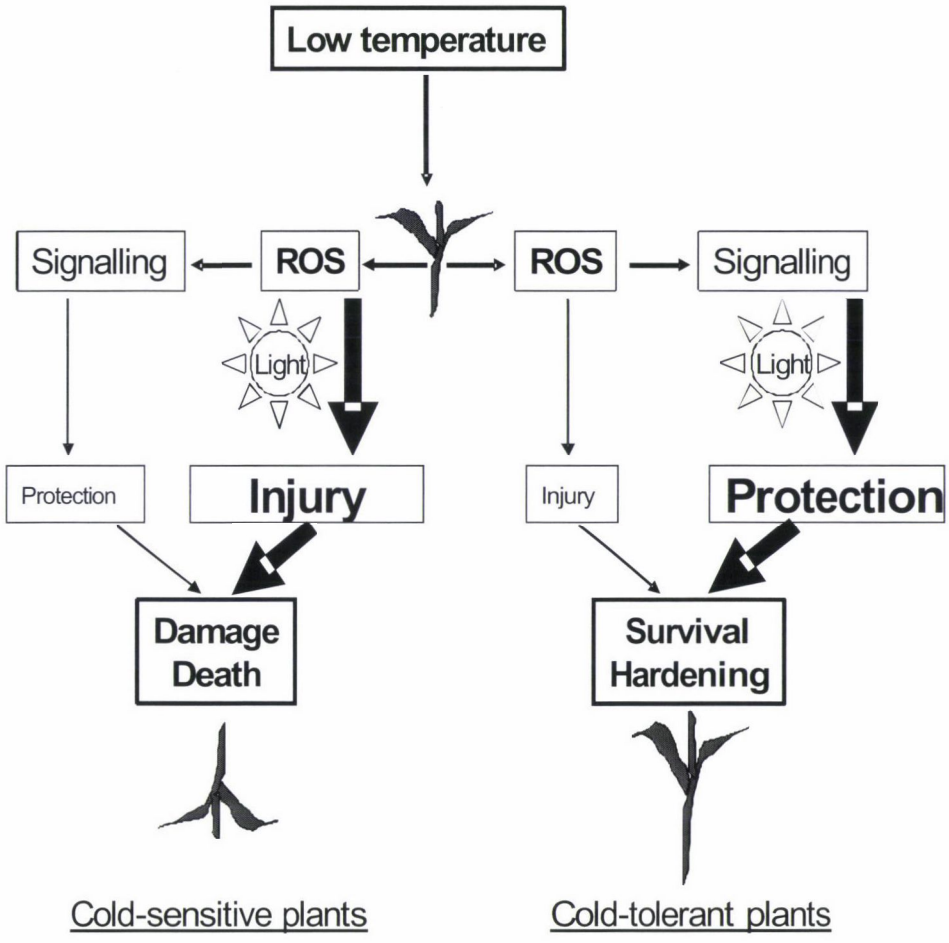

Fig. 2. Schematic representation of the role of ROS in cold-sensitive and cold-tolerant plants. ROS may directly induce damage; however, they may also induce signalling processes, which may lead to the development of acclimation mechanisms. Light plays a dual role. It contributes to photoinhibitory damage, and also induces acclimation processes. The balance between the damaging effects and the acclimatory processes will determine the level of injury and the acclimation processes

components, and can cause protein denaturation (Thomashow, 1999; Langridge et al., 2006). Due to excessive excitation of the respiratory and photosynthetic electron transport systems, growth at low temperature may increase the concentration of ROS. Because rates of synthetic reactions seem to be reduced in cold environments, demand for ATP may decrease. Under such conditions, a surplus of electrons at particular sites in the electron transport system may also occur, with resultant leakage of electrons. The leaked electrons may react with oxygen in the cell and generate ROS.

Even in freezing-tolerant winter varieties a certain period of growth at low, but non-freezing temperature is required for the development of frost hardiness and to fulfil the vernalization requirement, which ensures the correct timing of the vegetative/generative transition and protects reproductive organs from freezing injury. Resistance to low freezing temperatures is therefore a characteristic trait of tolerant plant species, acquired after a period of cold hardening. The cold acclimation of wheat plants includes changes in a wide range of physical and biochemical processes that allow functioning at low temperatures, such as the induction of antifreeze proteins (Yeh et al., 2000), changes in the membrane composition (Huner et al., 1987; Szalai et al., 2001), the accumulation of osmoprotectants (Konstantinova et al., 2002), polyamines (Rácz et al., 1996), etc., or changes in the redox status of plants (Szalai et al., 2009a), which may also lead to improved antioxidant capacity (Janda et al., 2003). To cope with exposure to low temperature, plants have developed various mechanisms to protect cellular activities and maintain whole plant integrity. Many stress-induced genes have been identified, including those encoding enzymes related to the scavenging of ROS (Gulick et al., 2005).

Several studies have been carried out on changes in the antioxidant activities of plants under unfavourable conditions, including low temperature stress; however, their role in the development of chilling and freezing tolerance is not clearly understood.

Chilling-induced oxidative stress

Chilling is a serious problem during the early development of maize and rice seedlings. It not only affects the growth of the plants, but also has a significant influence on the quality and quantity of the yield. An important consequence of chilling is the induction of oxidative stress due to the imbalance between the production and removal of ROS and the subsequent activation of antioxidants. Oxidative stress was indicated by the accumulation of H_2O_2 in chilled maize (Prasad et al., 1994a). The pre-treatment of maize seedlings with H_2O_2 or menadione, a superoxide-generating compound, at optimal growth temperature induced chilling tolerance (Prasad et al., 1994a). It was suggested that H_2O_2 had a dual effect at low temperatures: 1) During acclimation its early accumulation activates antioxidant enzymes such as catalase3 and guaiacol peroxidase. 2) In non-acclimated seedlings it accumulates to toxic concentrations due to the low level of antioxidants.

The ascorbate-glutathione cycle plays an important role in the regulation of the H_2O_2 concentration. The increase in the total glutathione (TG, GSH + GSSG) concentration observed in maize and rice during chilling (Kocsy et al., 1996; Guo et al., 2006) may be the result of its elevated synthesis, as shown by the greater incorporation of ^{35}S from labelled sulphate into GSH in maize (Kocsy et al., 1996). In addition, the γ-glutamylcysteine synthetase (γECS) activity and γ-glutamyl-

cysteine (γEC) level in the bundle sheet cells of maize were increased by chilling (Gómez et al., 2004). Under stress conditions the TG content may also be affected by the removal of the products of lipid peroxidation in the form of GS-conjugates (Mauch and Dudler, 1993). The activity of GSH S-transferase (GST), which catalyses this reaction, did not change during chilling in maize, but lost its circadian rhythm (Kocsy et al., 1997).

The role of GSH in the chilling tolerance of maize was proved by inducing a gradual decrease or increase in TG concentration using compounds modifying GSH synthesis (Kocsy et al., 2000a; 2001a, c). In these experiments a correlation was found between changes in the studied antioxidants (GSH concentration, GR and GST activities) and chilling tolerance (visible injuries, shoot and root length). The results also indicated that changes in TG concentration had a role in the regulation of GR activity and were independent of ABA (Kellős et al., 2008; Szalai et al., 2009a).

The maintenance of a high GSH/GSSG ratio is even more important for the efficient reduction of chilling-induced injuries than an increase in the TG content. Consistent with this assumption, the GSH/GSSG ratio was generally higher in chilling-tolerant maize genotypes than in sensitive ones (Hodges et al., 1996). A high GSH/GSSG ratio can be ensured by a sufficiently high level of glutathione reductase (GR) activity. In field experiments a greater increase in the amount of TG, accompanied by greater GR activity, was indeed observed in chilling-tolerant maize lines compared to sensitive ones during periods of low temperature in spring (Leipner et al., 1999). Besides the increase in the total GR activity, the appearance of new, stress-specific isoenzymes may be important in the adaptation to low temperature, as shown during chilling in the roots of a chilling-tolerant maize genotype (Pinhero et al., 1997).

GSH may be involved in the response to chilling together with thioredoxin h (Trx h), which is reduced by GSH. Trx h may form a conjugate with GSH (Casagrande et al., 2002) and may interact with the redox regulatory pathways described by Foyer and Noctor (2009). The role of Trx h in adaptation to chilling was demonstrated in maize, since its level increased in the chilling-tolerant genotype Z7 and decreased in the sensitive Penjalinan in response to chilling (Kocsy et al., 2004a). The interaction between GSH and Trx h systems was confirmed by the parallel changes in their levels both at low and high temperatures (Kocsy et al., 2004a). Although treatment with ABA increased the amount of Trx h in both Z7 and Penjalinan, and the amount of ABA increased during chilling in both genotypes, the Trx h level was only increased by chilling in Z7, while it was reduced in Penjalinan (Kocsy et al., 2004a). This indicates that the ABA-independent regulation of Trx h level also exists, as reported by Ishitani et al. (1997) in the case of low temperature-induced ABA-dependent and independent signalling pathways.

Besides the ascorbate-GSH cycle, other antioxidants are also important in the response to chilling. Thus, the activity of superoxide dismutase and catalase exhibited a great increase following chilling in chilling-tolerant rice genotypes (Guo et al., 2006). The overexpression of catalase increased chilling tolerance in

rice (Uemura et al., 2003). Maize seedlings were successfully acclimated to chilling by pre-exposure to 4°C for 1 d, an effect based on the activation of catalase, since the inhibition of this enzyme rendered the plants more sensitive again (Prasad, 1997). Although acclimation was mainly due to the activation of catalase, the activity of GR and guaiacol peroxidase was also induced. However, superoxide dismutase and ascorbate peroxidase were not affected by acclimation, which may indicate that it is not their activity levels that limit the removal of ROS during chilling. The alterations in the isozyme profiles of catalase, peroxidase and GR in maize may be even more important for effective acclimation than changes in the total activity of the antioxidant enzymes (Anderson et al., 1995).

An important component of adaptation to low temperature stress is the accumulation of compatible solutes (carbohydrates, free amino acids) which can stabilize the cellular osmotic pressure. The chilling-induced increase in the sucrose level was greater in a chilling-tolerant maize inbred line than in a sensitive one, but in the case of starch the elevation was more pronounced in the tolerant one in a growth chamber experiment (Sowiński et al., 1999). A tolerant rice genotype accumulated galactose and raffinose in response to chilling, whereas these saccharides declined in a sensitive one (Morsy et al., 2007). Similarly to the carbohydrates, the free amino acid contents also increased in maize during chilling and this change was greater in the light than in the dark (Szalai et al., 1997). Transgenic maize lines accumulated significantly higher levels of glycinebetaine than wild types and had increased chilling tolerance (Quan et al., 2004). Pro was found to increase chilling tolerance in maize, but by a different mechanism than ABA (Xin and Li, 1993). The increase of total free amino acids was mostly due to the accumulation of Ala, Glu, Gly and Ser, which might play an important role in the osmoregulation in maize under stress conditions (Quan et al., 2004). Various low temperature treatments caused complex changes in the profiles of the N-transporting amino acids Asp, Glu, Asn and Gln in maize (Simonovic and Anderson, 2007). In addition, chilling caused a reduction in glutamine synthetase activity, while acclimation protected it (Simonovic and Anderson, 2007).

The involvement of polyamines in the response to chilling was shown in various experimental systems. Besides their regulatory role, their protective effect may be due to their antioxidant function. Exogenous putrescine (Put) led to a recovery of the re-growth ability of chilled rice roots (Lee, 1997). The expression of the gene encoding spermidine (Spd) synthase 2 was up-regulated by chilling in rice and a similar effect was recorded for ABA (Imai et al., 2004). Chilling injuries in maize were generally correlated with the Spd concentration in the roots, the Put and Spd concentrations in the mesocotyl, and the Spd and spermine (Spm) concentrations in the coleoptile (Gao et al., 2009). Chilling induced an increase in the Put and Spd contents (Szalai et al., 1997). The beneficial effect of polyamines on the induction of chilling tolerance was also shown in maize suspension culture (Songstad et al., 1990). In maize the alternative pathway of polyamine synthesis catalysed by arginine decarboxylase proved to be important for chilling tolerance (Pál and Nagy, 2002). Spd and Spm play important roles in the imbibitional chill-

ing tolerance of maize seeds, probably through the prevention of chilling-induced MDA accumulation (Zheng et al., 2009).

Certain plant hormones also have an important role during chilling stress. Thus, the chilling tolerance of hydroponically grown maize seedlings was successfully increased by salicylic acid (Janda et al., 1999) or certain related compounds (Janda et al., 2000). This effect is based on an increase in the H_2O_2 concentration due to the specific inhibition of catalase-1 (Horváth et al., 2002). Chilling-induced SA accumulation was observed, particularly that of conjugated SA, in both the leaves and roots of two rice cultivars. No beneficial effect of SA treatment in protecting rice seedlings from chilling injury was observed at any concentration in either cultivar. In fact, pre-treatment with SA decreased their chilling tolerance, as confirmed by increased electrolyte leakage and lipid peroxidation. Furthermore, most of the activities of antioxidant enzymes decreased or remained unchanged after chilling in the leaves and roots of seedlings pre-treated with SA. These results implied that the down-regulation of the antioxidant defence system might be involved in the reduction of chilling tolerance in plants pre-treated with SA (Wang et al., 2009). A reduction in the SA level through the overexpression of the gene encoding one enzyme of its degradation resulted in a lower GSH concentration and decreased tolerance to oxidative stress in rice (Kusumi et al., 2006). SA treatment alone caused a significant increase in Put content and a decrease in Spm content in maize, while the Spd level only increased when the addition of SA was followed by low temperature stress (Németh et al., 2002). Another stress hormone, abscisic acid (ABA), also has a central role in the response of plants to chilling (Janowiak et al., 2002), although no conclusive evidence for the mediation of the acclimation process by ABA was found in maize seedlings (Anderson et al., 1994). Chilling resulted in a fast increase in the ABA level and a decrease in stomatal conductance, leaf water potential and root hydraulic conductance in maize, but after 24 h the leaf water potential recovered to control levels due to the stomatal control of transpiration and increased root hydraulic conductance (Melkonian et al., 2004). Lee et al. (1997) suggested that the effect of ABA is mediated by increased arginine decarboxylase activity and consequently by higher Put content in rice. ABA influenced the GR activity in rice cytosol, which may affect stress tolerance through the altered capacity of the ascorbate-glutathione cycle for the removal of H_2O_2 (Kaminaka et al., 1998).

Besides stress hormones, certain secondary messengers, like Ca^{2+}, are also involved in the response to low temperature stress. An interaction between Ca^{2+} and ROS was observed during the induction of the antioxidant system by ABA in maize, and it was concluded that Ca^{2+} may be located both before and after ROS in the signalling pathways related to oxidative stress (Jiang and Zhang, 2003). Yang and Poovaiah (2002) assumed a dual role for Ca^{2+} in the regulation of H_2O_2 homeostasis: (a) in the case of positive control H_2O_2 is produced due to the activation of NADPH oxidase; (b) during negative regulation the H_2O_2 concentration is reduced because of the activation of catalase.

During cold acclimation, coordinated changes take place in plants at the transcriptome, proteome and metabolome levels. Cold-induced transcriptome changes have been investigated in rice, resulting in the discovery of genes which may be involved in the acclimation process (Rabbani et al., 2003). Comparative proteomic analysis of chilled rice showed changes in the amounts of proteins involved in signal transduction, RNA processing, translation, protein processing, redox homeostasis, photosynthesis, photorespiration and the metabolism of carbon, nitrogen, sulphur and energy, but mRNA levels were not well correlated with the protein levels, as shown by quantitative real time PCR in the case of 44 different proteins (Yan et al., 2006). The analysis of cold-induced changes in gene expression, protein and metabolite pattern may result in the discovery of co-ordinated changes leading to effective adaptation to suboptimal temperatures.

Low temperature-induced oxidative stress in cold-tolerant plants

Exposure to low temperature may increase the amount of ROS not only in freezing-sensitive, but also in freezing-tolerant plants. A rapid, transient increase in the H_2O_2 level was detected in wheat plants after cold treatment by several authors; however, the level and the period of the peak of this increase varied in the different experiments. H_2O_2 readily permeates membranes and it is therefore not compartmentalised in the cell. After cold treatment of winter wheat seedlings the concentration of H_2O_2 in the leaves increased to about three times the control level within a few minutes, and returned to the normal level in 15 to 20 minutes (Okuda et al., 1991). In another study, although the H_2O_2 content and electrolyte leakage in wheat seedlings did not increase significantly within the first 12 h of cold treatment at 4°C, they reached peak values after 1 day and then declined again (Feng et al., 2008). The role of H_2O_2 in the cold acclimation of wheat was demonstrated by the comparison of wheat genotypes with different levels of freezing tolerance, since there was a correlation between cold acclimation-induced H_2O_2 accumulation and freezing tolerance (Soltész et al., 2011). The H_2O_2 content in freezing-stressed barley leaves also significantly increased after cold acclimation, but did not show a further increase during freezing treatment and recovery. However, in non-acclimated plants, the H_2O_2 content showed a significant increase during freezing and recovery (Dai et al., 2009). Further evidence for the role of H_2O_2 and the antioxidant system in the development of freezing tolerance was provided by an experiment where it was shown that UV-B irradiation increased freezing tolerance in winter wheat seedlings. H_2O_2 also increased rapidly after UV-B exposure, as did the activity of SOD. After recovery from freezing stress, there were increased activities of protective antioxidant enzymes, as well as higher concentrations of antioxidant compounds in UV-B irradiated leaves, compared with the control without UV-B irradiation (Yang et al., 2007). Pre-exposure of plants to H_2O_2 may elicit several changes in the metabolism, which affect the responses of plants to suboptimal temperature (Yu et al., 2003).

Low temperature or H_2O_2 treatment alone or combined significantly enhanced the level of the alternative respiratory pathway and the expression of alternative oxidase (Feng et al., 2008), suggesting that the alternative oxidase pathway may also be involved in the H_2O_2-induced changes in the metabolism. Enhanced AOX1 transcription was also observed in *Arabidopsis* cell cultures treated with NO, but neither catalase nor SOD were among the most strongly induced genes (Huang et al., 2002). These results indicated that the AOX pathway may also be an important part of the mechanism induced by exogenous oxidative shock.

The introduction of H_2O_2 into *Petunia hybrida* cells resulted in an increase in CN-resistant respiration and the expression of alternative oxidase protein, both of which remained present in the cells for several days, in spite of the fact that previous studies indicated that the half-life of H_2O_2 in cell cultures is much shorter, varying from 2–5 min to 1 h (Neill et al., 2002). It seems that the AOX pathway can be maintained in cells for days without increasing the major pool of endogenous H_2O_2.

Several non-enzymatic antioxidants participate in protection against the oxidative damage caused by various stress factors, including low temperature. The measurement of abiotic stress-induced changes in GSH levels, and their comparison in wheat genotypes with different stress tolerance, gave the first indication of the participation of GSH in the stress response (Kocsy et al., 2000b). The cold acclimation of cereals increased the levels of AA and GSH (Dai et al., 2009; Szalai et al., 2009a). In winter wheat the amounts of H_2O_2 and AA and the AA/DHA ratio showed a rapid and transient increase in the crown during the first week of acclimation, followed by a gradual increase during the subsequent 2 weeks. The amount of GSH and its ratio compared to GSSG quickly decreased during the first day, while later these parameters showed a continuous slow increase. The H_2O_2, AA and GSH concentrations and the AA/DHA and GSH/GSSG ratios were correlated with the level of freezing tolerance after 22 days at 2°C; hence, these parameters may have an important role in the acclimation process (Soltész et al., 2011).

The protective and regulatory roles of AA and GSH are based on changes in their redox state, which is defined by the reducing capacity of AA and GSH (AA and GSH concentration) and the half-cell reduction potential of the AA/DHA and GSH/GSSG couples (Schafer and Buettner, 2001). The redox state of AA and GSH differs in various organs, tissues, cells and compartments and also changes during the growth and development of the plants. The concentration of AA is one magnitude greater than that of GSH, so GSH is limiting for the maintenance of the redox state through the AA-GSH cycle. While glutathione reductase (GR) uses NADPH to reduce GSSG to GSH, various free radicals and oxidants are able to oxidize GSH to GSSG. The proportion of glutathione in the reduced form reflects the relative rates of reduction and oxidation and is always greater than 0.9 under non-stress conditions. Since the concentration of GSH in the chloroplast stroma is thought to be close to 5 mM, the reduced form of glutathione may act as an impor-

tant redox buffer, preventing enzyme inactivation by protecting potentially susceptible protein thiol groups (Noctor and Foyer, 1998).

Besides changes in the amount of non-enzymatic antioxidants, alterations in the activities of antioxidant enzymes also contribute to improved freezing tolerance. Cold-hardened plants grown at low temperature exhibited higher activities of antioxidant enzymes and increased amounts of non-enzymatic antioxidant compounds. In cereals a correlation was found between oxidative stress and the development of tolerance to freezing, suggesting that freezing tolerance at the subcellular level may be influenced by the ability to detoxify activated forms of oxygen (Bridger et al., 1994). While non-significant changes in POD or APX activities in response to cold hardening or freezing treatments were found in wheat plants, transferring the frozen cultivars to optimal growth conditions led to a dramatic increase (Apostolova et al., 2008). Freezing stress appeared to be a greater challenge for the spring cultivar, particularly in relation to plasma membrane intactness. An obvious stress response, more intense in spring wheat, could be detected based on the results obtained for enzyme activities and levels of lipid peroxidation and H_2O_2 (Apostolova et al., 2008). When various cereal species with different levels of freezing tolerance were compared, the highest correlation between enzyme activity and freezing tolerance was found in the case of POD and APX in hardened leaves. Neither enzyme activities from the crown nor those from unhardened leaves showed a significant positive correlation (Janda et al., 2003). Interestingly, POD isolated from winter oat showed almost zero activities in both leaf and crown. While the POD activity is low, the GST activity is relatively high in oat both in the leaf and root, compared with other cereal species. These results suggest that most of the antioxidant enzymes may play a role in the development of frost tolerance in cereals. However, the responses of these enzymes to the hardening conditions are different in the various species.

In contrast to the changes observed in the leaves or crown of different cereal species, the glutathione peroxidise (GPX) activity in barley root tips was not affected after exposure to cold, heat or drought treatments and only a slight increase was observed after H_2O_2 treatment (Halušková et al., 2009). Whereas in animals GPXs function as key enzymes that scavenge H_2O_2, in plants this function mainly belongs to catalases and the enzymes of the ascorbate–glutathione cycle. However, several authors have reported that ascorbate peroxidases are inhibited during severe and persistent stresses, so GPXs or GST/GPXs probably become the main H_2O_2 scavenging enzymes (Gueta-Dahan et al., 1997). Apart from salt treatment, most stresses (heat, drought and H_2O_2) caused only a slight increase in the activity of GST isolated from barley root tips, while its activity decreased slightly during cold treatment. The differences detected in the spatial distribution of GST and GPX activity along the root tip suggest that at least two proteins are responsible for these activities. These proteins play a crucial role in the differentiation processes of the root tip not only during stress, but also in unstressed seedlings. The use of various inhibitors suggests that the majority of the activities de-

tected in barley root tips are probably catalysed by GSTs that also possess GPX activity (Halušková et al., 2009).

In contrast to peroxidase enzymes a significant decrease in the catalase activity could be observed in the leaves of cold-hardened plants, while in the crown it did not change significantly (Janda et al., 2003; Plazek and Zur, 2003; Apostolova et al., 2008). There may be several explanations for this. It was earlier reported that catalase may suffer photo-oxidative damage in non-hardened winter rye leaves when the plants are exposed to low temperature (Streb et al., 1999). The occurrence of photoinhibition during low temperature hardening was reported by several authors (Hurry and Huner, 1991; Hurry et al., 1992; Janda et al., 1994a), but it is not generally directly connected with the development of freezing tolerance, as found earlier using chromosome substitution wheat lines (Janda et al., 1994a).

The plant hormone SA plays an important role in the stress response of plants. An increase in salicylic acid (SA) and its related compound *ortho*-hydroxycinnamic acid (*o*HCA) during low temperature hardening was reported in winter wheat plants (Janda et al., 2007). SA was also shown to inhibit a substantial portion of the catalase activity in several plant species (Raskin, 1992; Sánchez-Casas and Klessig, 1994). Salicylic acid plays an important role in the defence mechanisms against pathogen attack. It acts as a signal molecule in the development of systemic acquired resistance and mediates the oxidative burst in the hypersensitive reaction leading to cell death (Raskin, 1992). In recent years the role of salicylic acid and its related compounds has been widely investigated not only in biotic but also abiotic stresses. SA was found to accumulate during exposure to ozone or UV light (Yalpani et al., 1994; Sharma et al., 1996). It may ameliorate the damaging effects of heavy metals in rice (Mishra and Choudhuri, 1999) and improve the heat-shock tolerance of mustard (Dat et al., 1998a,b) and tobacco plants (Dat et al., 2000). Acetyl SA induced heat tolerance in potato microplants (Lopez-Delgado et al., 1998) and decreased the inhibitory effect of drought (Hamada, 1998) and salt stress (Al-Hakimi and Hamada, 2001) in wheat.

The synthesis of SA from benzoic acid is catalysed by the benzoic acid 2-hydroxylase enzyme (Ribnicky et al., 1998). It was also shown that not only SA, but also its derivative acetyl-SA (aspirin) or its putative precursors benzoic acid or *o*HCA caused a decrease in catalase activity (Janda et al., 2000; Horváth et al., 2002). The ability of SA and related compounds to inhibit catalase activity was shown not only *in vivo*, but also *in vitro,* isolated from maize plants (Horváth et al., 2002). Other results confirmed the connection between the action of SA and changes in the antioxidant activities in wheat, where exogenous SA may be involved in cold tolerance by regulating apoplastic proteins, including antifreeze proteins and antioxidant enzyme activities (Tasgin et al., 2007). However, the role of endogenous SA during the development of frost tolerance is still poorly understood.

Besides SA, ABA is considered to be one of the most important hormones involved in the plant response to frost stress. Two kinds of evidence support a role

for ABA in the acclimation of plants to low temperature. Firstly, endogenous ABA levels rise in several species when they are exposed to stress conditions (Veisz et al., 1996; Lee et al., 1997; Janowiak et al., 2002); secondly, the application of ABA induced freezing tolerance in wheat (Veisz et al., 1996). The protective mechanism of ABA against low temperature stress is linked to its capacity for stabilising the water status by increasing root hydraulic conductivity and by closing the stomata, although ABA also induces antioxidant enzymes and modulates polyamine levels (Xin and Li, 1992; Prasad et al., 1994b; Lee et al., 1997; Aroca et al., 2001; Jiang and Zhang, 2002). A recent study on chickpea plants showed that the effects of cold stress were partly overcome by ABA treatment because of the improvement in the water status of the leaves as well as the greater activities of superoxide dismutase, catalase and ascorbate peroxidase, and the higher amounts of ascorbic acid, glutathione and proline in these plants (Kumar et al., 2008). Exogenous ABA treatment was also shown to increase the endogenous production of H_2O_2 (Pei et al., 2000). Jiang and Zhang (2002) reported that ABA accumulation triggered the increased generation of ROS, which consequently regulated the activity of antioxidant enzymes. However, Ohtsu et al. (2002) found that ABA treatment did not induce AOX1 transcription in rice leaves. These results suggest that H_2O_2 could initiate the signalling for the induction of the AOX pathway; however, ROS are not necessary for the maintenance of long-term AOX activity, and the signal pathway of the alternative pathway might be independent of the pool of endogenous H_2O_2.

The antioxidative system, including the GSH/GSSG redox couple, may have evolved for the adjustment of the cellular redox state and redox signalling and for the orchestration of gene expression (Noctor and Foyer, 1998). Several regulatory and structural genes controlled by the thiol-disulphide status and ROS signalling have been identified using transcript profiling in mutant and transgenic *Arabidopsis* and in wild-type plants treated with dithiothreitol or ROS-generating agents, which could clarify the function of the redox network (Gadjev et al., 2006; Kolbe et al., 2006). This network controls the level of ROS by integrating signals from different cell compartments during abiotic stress, and the GSH/GSSG couple participates in its fine tuning (Meyer, 2008). The comparative transcriptome analysis of cold-hardened wheat revealed that the expression of several genes involved in the adaptation to low temperature, including the transcript levels of those encoding antioxidants, exhibited a greater cold-induced increase in freezing-tolerant wheat genotypes than in sensitive ones (Kocsy et al., 2010).

Role of light in cold-induced oxidative damage

The rate of cold-induced damage depends not only on the temperature, but also on other environmental factors, especially irradiance. Plants often harvest more light than they can use in photosynthesis. The exposure of plants to a light intensity higher than that which can be utilized in the photosynthetic processes

may cause damage to the photosynthetic apparatus. This phenomenon is called photoinhibition. Due to the rapid decline in the efficiency of light utilization, especially in chilling-sensitive plants such as maize, photoinhibition may be a substantial part of the chilling injury (Janda et al., 1994b). The major site of photoinhibition is the Photosystem 2 (PS 2) complex, in which electron transport is inhibited and protein structure is damaged. Various ROS formed from the molecular oxygen produced as a by-product of water oxidation are involved in the light-induced damage of PS 2. For a recent review of the mechanism of photoinhibition, see Vass and Cser (2009).

Besides the damaging role of light during exposure to low temperature, results show that light intensity is a key factor in the development of freezing tolerance during the frost hardening of wheat plants. It has long been known that the frost hardening of cereals is more effective under normal light conditions than in the dark (Gray et al., 1997; Apostol et al., 2006). The decisive role of light in the development of freezing tolerance was also indicated by the improvement in freezing tolerance under elevated light conditions (Gray et al., 1997). The freezing tolerance induced by high light at normal, non-hardening temperatures was more pronounced in winter wheat varieties than in spring ones (Szalai et al., 2009b). Light serves as a driving force for photosynthesis, the main energy source in plants. The induction of certain cold-stimulated genes in wheat has been shown to be correlated with the relative reduction state of PS 2 rather than with growth temperature or growth irradiance *per se* (Gray et al., 1997). Transcriptions of certain nuclear genes are orchestrated by photosynthetic products. Hardening temperatures in the light may increase the activity of the cyclic photosynthetic electron transport chain, which may contribute to an optimal energy balance during periods of low temperature stress (Apostol et al., 2006). Recent results also showed that, besides the changes in the PS 2-related electron transport processes, several other mechanisms, including the lipid, polyamine or salicylic acid metabolisms and the altered antioxidant activity may contribute to light-induced freezing tolerance (Janda et al., 2007; Szalai et al., 2009b). The greatest induction of certain antioxidant enzymes in winter wheat leaves, for example glutathione reductase or ascorbate peroxidase, occurred when the cold treatment was carried out in the light, but high light intensity at normal, non-hardening temperature also increased the activity of these enzymes (Janda et al., 2007).

Brachypodium as a model plant to study the response to low temperature stress

Brachypodium distachion L. *(Brachypodium)* is an optimal model plant for studying the response of cereals to low temperature stress. During the evolution of the *Pooideae* it diverged just prior to the clade of "core pooid" genera to which most temperate cereals and forage grasses belong (reviewed by Draper et al., 2001). *Brachypodium* is very similar to *Arabidopsis* in many aspects of its biol-

ogy, such as height, planting density, breeding system, duration of the life cycle, genome size and chromosome number. *Brachypodium* can be used as a complementary model to rice, and it has several advantages, such as smaller genome size, fewer chromosomes (2n = 10), shorter life cycle, lower height, larger planting density and simpler cultivation.

Brachypodium has only yet been used for the investigation of the effect of abiotic stresses, including that of low temperature, in a few cases. Cold treatment resulted in significant changes in the expression of three conserved microRNAs (miRNAs) and 25 predicted miRNAs (Zhang et al., 2009). These miRNAs are endogenous small RNAs having large-scale regulatory effects on plant development and stress responses. The results published on miRNAs in cold-treated *Brachypodium* are very important, since little is known about their involvement in the response to low temperature stress in winter-habit monocots. Substantial phenotypic variation in drought tolerance was revealed by a comparison of 57 natural populations of *Brachypodium* (Luo et al., 2011). Variation can also be assumed for freezing tolerance, since water shortage is an important component of low temperature stress. The cited studies indicate that *Brachypodium* will prove to be a powerful tool for the study of cold acclimation and freezing tolerance in the future.

Conclusions

Low temperature stress-induced changes in the ROS level activate the antioxidant system, altering the redox state of the cells and activating various signalling pathways and defence mechanisms, leading to acclimation. Excessive stress may lead to cell death. Both acclimation and death processes are connected to complex, coordinated changes at the transcriptome, proteome and metabolome levels. The molecular and cell biological analysis of these alterations may result in the discovery of new, unknown mechanisms involved in adaptation to low temperature stress. The use of model plants such as *Brachypodium* will facilitate this work.

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References

Al-Hakimi, A. M. A., Hamada, A. M. (2001): Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamin or sodium salicylate. *Biol. Plant.*, **44**, 253–261.

Alscher, R. G., Donaue, J. L., Cramer, C. L. (1997): Reactive oxygen species and antioxidants: relationships in green cells. *Physiol. Plant.*, **100**, 224–233.

Anderson, M. D., Prasad, T. K., Stewart, C. R. (1995): Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiol.,* **109**, 1247–1257.

Anderson, M. D., Prasad, T. K., Martin, B. A., Stewart, C. R. (1994): Differential gene expression in chilling-acclimated maize seedlings and evidence for the involvement of abscisic acid in chilling tolerance. *Plant Physiol.,* **105**, 331–339.

Apel, K., Hirt, H. (2004): Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.,* **55**, 373–399.

Apostol, S., Szalai, G., Sujbert, L., Popova, L. P., Janda, T. (2006): Non-invasive monitoring of the light-induced cyclic photosynthetic electron flow during cold hardening in wheat leaves. *Z. Naturforsch. C.,* **61c**, 734–740.

Apostolova, P., Yordanova, R., Popova, L. (2008): Response of antioxidative defence system to low temperature stress in two wheat cultivars. *Gen. Appl. Plant Physiol.,* Spec. Issue **34**, 281–294.

Aroca, R., Tognoni, F., Irigoyen, J. J., Sánchez-Díaz, M., Pardossi, A. (2001): Different root low temperature responses of two maize genotypes differing in chilling sensitivity. *Plant Physiol. Biochem.,* **39**, 1067–1073.

Aslund, F., Beckwith, J. (1999): Bridge over troubled waters: sensing stress by disulfide bond formation. *Cell,* **96**, 751–753.

Bridger, G. M., Yang, W., Falk, D. E., McKersie, B. D. (1994): Cold-acclimation increases tolerance of activated oxygen in winter cereals. *J. Plant Physiol.,* **144**, 235–240.

Cakmak, I., Marschner, H. (1992): Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiol.,* **98**, 1222–1227.

Casagrande, S., Bonetto, V., Fratelli, M., Gianazza, E., Eberini, I., Massignan, T., Salmona, M., Chang, G., Holmgren, A., Ghezzi, P. (2002): Glutathionylation of human thioredoxin: A possible crosstalk between the glutathione and thioredoxin systems. *Proc. Nat. Acad. Sci. USA,* **99**, 9745–9749.

Dai, F., Huang, Y., Zhou, M., Zhang, G. (2009): The influence of cold acclimation on antioxidative enzymes and antioxidants in sensitive and tolerant barley cultivars. *Biol. Plant.,* **53**, 257–262.

Dat, J. F., Foyer, C. H., Scott, I. M. (1998b): Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. *Plant Physiol.,* **118**, 1455–1461.

Dat, J. F., Lopez-Delgado, H., Foyer, C. H., Scott, I. M. (1998a): Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiol.,* **116**, 1351–1357.

Dat, J. F., Lopez-Delgado, H., Foyer, C. H., Scott, I. M. (2000): Effects of salicylic acid on oxidative stress and thermotolerance in tobacco. *J. Plant Physiol.,* **156**, 659–665.

Draper, J., Mur, L. A. J., Jenkins, G., Ghosh-Biswas, G. C., Bablak, P., Hasterok, R., Routledge, A. P. M. (2001): *Brachypodium distachyon.* A new model system for functional genomics in grasses. *Plant Physiol.,* **127**, 1539–1555.

Feng, H., Li, X., Duan, J., Li, H., Liang, H. (2008): Chilling tolerance of wheat seedlings is related to an enhanced alternative respiratory pathway. *Crop Sci.,* **48**, 2381–2388.

Foyer, C. H., Halliwell, B. (1976): The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta,* **133**, 21–25.

Foyer, C. H., Noctor, G. (2005): Redox homeostasis and antioxidant signalling: A metabolic link between stress perception and physiological responses. *Plant Cell,* **17**, 1866–1875.

Foyer, C. H., Noctor, G. (2009): Redox regulation in photosynthetic organisms: Signalling, acclimation, and practical implications. *Antioxid. Redox Signal.,* **11**, 861–905.

Foyer, C. H., Descourvières, P., Kunert, K. J. (1994a): Protection against oxygen radicals: An important defense mechanism studied in transgenic plants. *Plant Cell Environ.,* **17**, 507–523.

Foyer, C. H., Lelandais, M., Kunert, K. J. (1994b): Photooxidative stress in plants – a special review. *Physiol. Plant.,* **92**, 696–717.

Foyer, C. H., Lopez-Delgado, H., Dat, J. F., Scott I. M. (1997): Hydrogen peroxide and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. *Physiol. Plant.,* **100**, 241–254.

Gadjev, I., Vanderauwera, S., Gechev T. S., Laloi, C., Minkov, I. N., Shulaev, V., Apel, K., Inzé, D., Mittler, R., van Breusegem, F. (2006): Transcriptomic footprints disclose specificity of reactive oxygen species signalling in *Arabidopsis*. *Plant Physiol.,* **141**, 436–445.

Gao, C. H., Hu, J., Zhang, S., Zheng, Y. Y., Knapp, A. (2009): Association of polyamines in governing the chilling sensitivity of maize genotypes. *Plant Growth Regul.,* **57**, 31–38.

Gómez, L. D., Vanacker, H., Buchner, P., Noctor, G., Foyer, C. H. (2004): Intercellular distribution of glutathione synthesis in maize leaves and its response to short-term chilling. *Plant Physiol.,* **134**, 1662–1671.

Gray, G. R., Chauvin, L.-P., Sarhan, F., Huner, N. P. A. (1997): Cold acclimation and freezing tolerance. A complex interaction of light and temperature. *Plant Physiol.,* **114**, 467–474.

Gueta-Dahan, Y., Yaniv, Z., Zilinskas, B. A., Ben-Hayyim, G. (1997): Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in Citrus. *Planta,* **203**, 460–469.

Gulick, P. J., Drouin, S., Yu, Z., Danyluk, J., Poisson, G., Monroy, A. F., Sarhan, F. (2005): Transcriptome comparison of winter and spring wheat responding to low temperature. *Genome,* **48**, 913–923.

Guo, Z., Ou, W., Lu, S., Zhong, Q. (2006): Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. *Plant Physiol. Biochem.,* **44**, 828–836.

Gupta, R., Luan, S. (2003): Redox control of protein tyrosine phosphatases and mitogen-activated protein kinases in plants. *Plant Physiol.,* **132**, 1149–1152.

Halušková, L., Valentovičová, K., Huttová, J., Mistrík, I., Tamás, L. (2009): Effect of abiotic stresses on glutathione peroxidase and glutathione S-transferase activity in barley root tips. *Plant Physiol. Biochem.,* **47**, 1069–1074.

Hamada, A. M. (1998): Effects of exogenously added ascorbic acid, thiamin or aspirin on photosynthesis and some related activities of drought-stressed wheat plants. pp. 2581–2584. In: Garab, G. (ed.), *Photosynthesis: Mechanisms and Effects.* Vol. 4. Kluwer Acad. Publ., Dordrecht.

Hodges, D. M., Andrews, C. J., Johnson, D. A., Hamilton, R. I. (1996): Antioxidant compound responses to chilling stress in differentially sensitive inbred maize lines. *Plant Physiol.,* **90**, 685–692.

Horváth, E., Janda, T., Szalai, G., Páldi, E. (2002): *In vitro* salicylic acid inhibition of catalase activity in maize: differences between the isoenzymes and a possible role in the induction of chilling tolerance. *Plant Sci.,* **163**, 1129–1135.

Horváth, E., Pál, M., Szalai, G., Páldi, E., Janda, T. (2007): Exogenous 4-hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants. *Biol. Plant.,* **51**, 480–487.

Huang, X., von Rad, U., Durner, J. (2002): Nitric oxide induces transcriptional activation of the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension cells. *Planta,* **215**, 914–923.

Huner, N. P. A., Krol, M., Williams, J. P., Maissan, E., Low, P. S., Roberts, D., Thompson, J. E. (1987): Low temperature development induces a specific decrease in trans-D3-hexadecenoic acid content which influences LHCII organization. *Plant Physiol.*, **84**, 12–18.

Hurry, V. M., Huner, N. P. A. (1991): Low growth temperature effects a differential inhibition of photosynthesis in spring and winter wheat. *Plant Physiol.*, **96**, 491–497.

Hurry, V. M., Krol, M., Öquist, G., Huner, N. P. A. (1992): Effect of long-term photoinhibition on growth and photosynthesis of cold-hardened spring and winter wheat. *Planta,* **188**, 369–375.

Imai, R., Ali, A., Pramanik, Md. H. R., Nakaminami, K., Sentoku, N., Kato, H. (2004): A distinctive class of spermidine synthase is involved in chilling response in rice. *J. Plant Physiol.*, **161**, 883–886.

Ishitani, M., Xiong, L., Stevenson, B., Zhu, J. K. (1997): Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis:* Interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. *Plant Cell,* **9**, 1935–1949.

Janda, T., Szalai, G., Tari, I., Páldi, E. (1999): Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (*Zea mays* L.) plants. *Planta,* **208**, 175–180.

Janda, T., Kissimon, J., Szigeti, Z., Veisz, O., Páldi, E. (1994a): Characterization of cold hardening in wheat using fluorescence induction parameters. *J. Plant Physiol.*, **143**, 385–388.

Janda, T., Szalai, G., Antunovics, Z., Horváth, E., Páldi, E. (2000): Effect of benzoic acid and aspirin on chilling tolerance and photosynthesis in young maize plants. *Maydica,* **45**, 29–33.

Janda, T., Szalai, G., Rios-Gonzalez, K., Veisz, O., Páldi, E. (2003): Comparative study of frost tolerance and antioxidant activity in cereals. *Plant Sci.*, **164**, 301–306.

Janda, T., Szalai, G., Kissimon, J., Páldi, E., Marton, C., Szigeti, Z. (1994b): Role of irradiance in the chilling injury of young maize plants studied by chlorophyll fluorescence induction measurements. *Photosynthetica,* **30**, 293–299.

Janda, T., Szalai, G., Leskó, K., Yordanova, R., Apostol, S., Popova, L. P. (2007): Factors contributing to enhanced freezing tolerance in wheat during frost hardening in the light. *Phytochemistry,* **68**, 1674–1682.

Janowiak, F., Maas, B., Dörffling, K. (2002): Importance of abscisic acid for chilling tolerance of maize seedlings. *J. Plant Physiol.*, **159**, 635–643.

Jiang, M., Zhang, J. (2002): Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *J. Exp. Bot.*, **53**, 2401–2410.

Jiang, M., Zhang, J. (2003): Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in abscisic acid-induced antioxidant defence in leaves of maize seedlings. *Plant Cell Env.*, **26**, 929–939.

Kaminaka, H., Morita, S., Nakajima, M., Masumura, T., Tanaka, K. (1998): Gene cloning and expression of cytosolic glutathione reductase in rice (*Oryza sativa* L.). *Plant Cell Physiol.*, **39**, 1269–1280.

Kellős, T., Tímár, I., Szilágyi, V., Szalai, G., Galiba, G., Kocsy, G. (2008): Stress hormones and abiotic stresses have different effects on antioxidants in maize lines with different sensitivity. *Plant Biol.*, **10**, 563–572.

Kendall, E. J., McKersie, B. D. (1989): Free radical and freezing injury to cell membranes of winter wheat. *Physiol. Plant.*, **76**, 86–94.

Kocsy, G., Galiba, G., Brunold, C. (2001b): Role of glutathione in adaptation and signalling during chilling and cold acclimation in plants. *Physiol. Plant.*, **113**, 158–164.

Kocsy, G., Owttrim, G., Brander, K., Brunold, C. (1997): Effect of chilling on the diurnal rhythm of enzymes involved in protection against oxidative stress in a chilling tolerant and a chilling sensitive maize genotype. *Physiol. Plant.*, **99**, 249–254.

Kocsy, G., Brunner, M., Rüegsegger, A., Stamp, P., Brunold, C. (1996): Glutathione synthesis in maize genotypes with different sensitivity to chilling. *Planta,* **198**, 365–370.

Kocsy, G., Kobrehel, K., Szalai, G., Duviau, M.-P., Buzás, Z., Galiba, G. (2004a): Thioredoxin *h* and glutathione as abiotic stress tolerance markers in maize. *Env. Exp. Bot.,* **52**, 101–112.

Kocsy, G., Szalai, G., Vágújfalvi, A., Stéhli, L., Orosz, G., Galiba, G. (2000b): Genetic study of glutathione accumulation during cold hardening in wheat. *Planta,* **210**, 295–301.

Kocsy, G., Tóth, B., Berzy, T., Szalai, G., Jednákovits, A., Galiba, G. (2001c): Glutathione reductase activity and chilling tolerance are induced by a hydroxylamine derivative BRX 156 in maize and soybean. *Plant Sci.,* **160**, 943–950.

Kocsy, G., von Ballmoos, P., Rüegsegger, A., Szalai, G., Galiba, G., Brunold, C. (2001a): Increasing the glutathione content in a chilling-sensitive maize genotype using safeners increased protection against chilling-induced injury. *Plant Physiol.,* **127**, 1147–1156.

Kocsy, G., Perovic, D., Himmelbach, A., Athmer, B., Schweizer, P., Galiba, G., Stein, N. (2010): Cold-induced transcript profile changes related to freezing tolerance in wheat – effect of chromosome 5A. *Mol. Genet. Genomics,* **283**, 351–363.

Kocsy, G., von Ballmoos, P., Suter, M., Rüegsegger, A., Galli, U., Szalai, G., Galiba, G., Brunold, C. (2000a): Inhibition of glutathione synthesis reduces chilling tolerance in maize. *Planta,* **211**, 528–536.

Kolbe, A., Oliver, S. N., Fernie, A. R., Stitt, M., van Dongen, J. T., Geigenberger, P. (2006): Combined transcript and metabolite profiling of *Arabidopsis* leaves reveals fundamental effects of the thiol-disulphide status on plant metabolism. *Plant Physiol.,* **141**, 412–422.

Konstantinova, T., Parvanova, D., Atanassov, A., Djilianov, D. (2002): Freezing tolerant tobacco, transformed to accumulate osmoprotectants. *Plant Sci.,* **163**, 157–164.

Kumar, S., Kaur, G., Nayyar, H. (2008): Exogenous application of abscisic acid improves cold tolerance in chickpea (*Cicer arietinum* L.). *J. Agr. Crop Sci.,* **194**, 449–456.

Kusumi, K., Yaeno, T., Kojo, K., Hirayama, M., Hirokawa, D., Yara, A., Iba, K. (2006): The role of salicylic acid in the glutathione-mediated protection against photooxidative stress in rice. *Physiol. Plant.,* **128**, 651–666.

Langridge, P., Paltridge, N., Fincher, G. (2006): Functional genomics of abiotic stress tolerance in cereals. *Briefings in Functional Genomics and Proteomics,* **4**, 343–354.

Lee, T. M. (1997): Polyamine regulation of growth and chilling tolerance of rice (*Oryza sativa* L.) roots cultured *in vitro*. *Plant Sci.,* **122**, 111–117.

Lee, T. M., Lur, H. S., Chu, C. (1997): Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings. II. Modulation of free polyamine levels. *Plant Sci.,* **126**, 1–10.

Leipner, J., Fracheboud, Y., Stamp, P. (1999): Effect of growing season on the photosynthetic apparatus and leaf antioxidative defenses in two maize genotypes of different chilling tolerance. *Environ. Exp. Bot.,* **42**, 129–139.

Lopez-Delgado, H., Dat, J., Foyer, C. H., Scott, I. M. (1998): Induction of thermotolerance in potato microplants by acetylsalicylic acid and H_2O_2. *J. Exp. Bot.,* **49**, 713–720.

Luo, N., Liu, J. X., Yu, X. Q., Jiang, Y. W. (2011): Natural variation of drought response in *Brachypodium distachyon*. *Physiol. Plant.,* **141**, 19–29.

Mauch, F., Dudler, R. (1993): Differential induction of distinct glutathione S-transferases of wheat by xenobiotics and pathogen attack. *Plant Physiol.,* **102**, 1193–1201.

Melkonian, J., Yu, L., Setter, T. (2004): Chilling responses of maize (*Zea mays* L.) seedlings: root hydraulic conductance, abscisic acid, and stomatal conductance. *J. Exp. Bot.,* **55**, 1751–1760.

Meyer, A. J. (2008): The integration of glutathione homeostasis and redox signalling. *J. Plant Physiol.,* **165**, 1390–1403.

Mishra, A., Choudhuri, M. A. (1999): Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. *Biol. Plant.,* **42**, 409–415.

Mittler, R. (2002): Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.,* **7**, 405–410.

Mittler, R., Vanderauwera, S., Gollery, M., Breusegem, F. V. (2004): Reactive oxygen gene network of plants. *Trends Plant Sci.,* **9**, 490–498.

Morsy, M. R., Jouve, L., Hausman, J. F., Hoffmann, L., Stewart, J. McD. (2007): Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *J. Plant Physiol.,* **164**, 157–167.

Neill, S. J., Desikan, R., Clarke, A., Hurst, R. D., Hancook, J. T. (2002): Hydrogen peroxide and nitric oxide as signaling molecules in plants. *J. Exp. Bot.,* **53**, 1237–1247.

Németh, M., Janda, T., Horváth, E., Páldi, E., Szalai, G. (2002): Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. *Plant Sci.,* **162**, 569–574.

Noctor, G., Foyer, C. H. (1998): Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **49**, 249–279.

Ohtsu, K., Ito, Y., Saika, H., Nakazono, M., Tsutsumi, N., Hirai, A. (2002): ABA-independent expression of rice alternative oxidase genes under environmental stresses. *Plant Biotechnol.,* **19**, 187–190.

O'Kane, D., Gill, V., Boyd, P., Burdon, R. (1996): Chilling, oxidative stress and antioxidant responses in *Arabidopsis thaliana* callus. *Planta,* **198**, 371–377.

Okuda, T., Matsuda, Y., Yamanaka, A., Sagisaka, S. (1991): Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. *Plant Physiol.,* **97**, 1265–1267.

Pál, M., Nagy, E. (2002): Chilling tolerance of maize. *Acta Agron. Hung.,* **50**, 95–106.

Pang, C. H., Zhang, S. J., Gong, Z. Z., Wang, B. S. (2005): NaCl treatment markedly enhances H_2O_2-scavenging system in leaves of halophyte *Suaeda salsa*. *Physiol. Plant.,* **125**, 490–499.

Pei, Z. M., Murata, N., Benning, G., Thomine, S., Klüsener, B., Allen, G. J., Grill, E., Schroeder, J. I. (2000): Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature,* **406**, 731–734.

Pinhero, R. G., Rao, M. V., Paliyath, G., Murr, D. P., Fletcher, R. A. (1997): Changes in activities of antioxidant enzymes and their relationship to genetic and paclobutrazol-induced chilling tolerance of maize seedlings. *Plant Physiol.,* **114**, 695–704.

Plazek, A., Zur, I. (2003): Cold-induced plant resistance to necrotrophic pathogens and antioxidant enzyme activities and cell membrane permeability. *Plant Science,* **164**, 1019–1028.

Polidoros, A. N., Scandalios, J. G. (1999): Role of hydrogen peroxide and different classes of antioxidants in the regulation of catalase and glutathione S-transferase gene expression in maize. *Physiol. Plant.,* **106**, 112–120.

Prasad, T. K. (1997): Role of catalase in inducing chilling tolerance in pre-emergent maize seedlings. *Plant Physiol.,* **114**, 1369–1376.

Prasad, T. K., Anderson, M. D., Stewart, C. R. (1994b): Acclimation, hydrogen peroxide, and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings. *Plant Physiol.,* **105**, 619–627.

Prasad, T. K., Anderson, M. D., Martin, B. A., Stewart, C. R. (1994a): Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. *Plant Cell,* **6**, 65–74.

Quan, R., Shang, M., Zhang, H., Zhao, Y. X., Zhang, J. (2004): Improved chilling tolerance by transformation with *betA* gene for the enhancement of glycinebetaine synthesis in maize. *Plant Sci.,* **166**, 141–149.

Rabbani, A., Maruyama, K., Abe, H., Khan, M. A., Katsura, K., Ito, Y., Yoshiwara, K., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K. (2003): Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol.,* **133**, 1755–1767.

Rácz, I., Kovács, M., Lásztity, D., Veisz, O., Szalai, G., Páldi, E. (1996): Effect of short-term and long-term low temperature stress on polyamine biosynthesis in wheat genotypes with varying degrees of frost tolerance. *J. Plant Physiol.,* **148**, 368–373.

Raskin, I. (1992): Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **43**, 439–463.

Ribnicky, D. M., Shulaev, V., Raskin, I. (1998): Intermediates of salicylic acid biosynthesis in tobacco. *Plant Physiol.,* **118**, 565–572.

Sánchez-Casas, P., Klessig, D. F. (1994): A salicylic acid-binding activity and a salicylic acid-inhibitable catalase activity are present in a variety of plant species. *Plant Physiol.,* **106**, 1675–1679.

Schafer, F. O., Buettner, G. R. (2001): Redox environment of the cell as viewed through the redox state of the glutathione disulphide/glutathione couple. *Free Rad. Biol. Med.,* **30**, 1191–1212.

Sharma, Y. J., León, J., Raskin, I., Davis, K. R. (1996): Ozone-induced responses in *Arabidopsis thaliana*: the role of salicylic acid in the accumulation of defense related transcripts and induced resistance. *Proc. Nat. Acad. Sci. USA,* **93**, 5099–5104.

Simonović, A. D., Anderson, M. D. (2007): Analysis of methionine oxides and nitrogen-transporting amino acids in chilled and acclimated maize seedlings. *Amino Acids,* **33**, 607–613.

Soltész, A., Tímár, I., Vashegyi, I., Tóth, B., Kellős, T., Szalai, G., Vágújfalvi, A., Kocsy, G., Galiba, G. (2011): Redox changes during cold acclimation affect freezing tolerance but not the vegetative/reproductive transition of the shoot apex in wheat. *Plant Biology, 13: no.* DOI: 10.1111/j.1438-8677.2010.00429.x

Songstad, D. D., Duncan, D. R., Widholm, J. M. (1990): Proline and polyamine involvement in chilling tolerance of maize suspension cultures. *J. Exp. Bot.,* **41**, 289–294.

Sowiński, P., Dalbiak, A., Tadeusiak, J., Ochodzki, P. (1999): Relations between carbohydrate accumulation in leaves, sucrose phosphate synthase activity and photoassimilate transport in chilling treated maize seedlings. *Acta Physiol. Plant.,* **21**, 375–381.

Streb, P., Shang, W., Feierabend, J. (1999): Resistance of cold-hardened winter rye leaves (*Secale cereale* L.) to photo-oxidative stress. *Plant Cell Environ.,* **22**, 1211–1223.

Suzuki, N., Mittler, R. (2006): Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. *Physiol. Plant.,* **126**, 45–51.

Szalai, G., Pap, M., Janda, T. (2009b): Light-induced frost tolerance differs in winter and spring wheat plants. *J. Plant Physiol.,* **166**, 1826–1831.

Szalai, G., Janda, T., Bartók, T., Páldi, E. (1997): Role of light in changes in free amino acid and polyamine contents at chilling temperature in maize *(Zea mays). Physiol. Plant.,* **101**, 434–438.

Szalai, G., Janda, T., Páldi, E., Dubacq, J.-P. (2001): Changes in the fatty acid unsaturation after hardening in wheat chromosome substitution lines with different cold tolerance. *J. Plant Physiol.,* **158**, 663–666.

Szalai, G., Kellos, T., Galiba, G., Kocsy, G. (2009a): Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. *J. Plant Growth Regul.,* **28**, 66–80.

Tasgin, E., Atici, Ö., Nalbantoglu, B., Popova, L. P. (2007): Effects of salicylic acid and cold treatments on protein levels and on the activities of antioxidant enzymes in the apoplast of winter wheat leaves. *Phytochemistry,* **67**, 710–715.

Thomashow, M. F. (1999): Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Ann Rev. Plant. Physiol. Plant Mol. Biol.,* **50**, 571–599.

Uemura, M., Onodera, H., Saruyama, H. (2003): Involvement of reactive oxygen species in chilling injury of rice leaves. *Cryobiology Cryotechnol.,* **49**, 215–220.

Vass, I., Cser, K. (2009): Janus-faced charge recombinations in photosystem II photoinhibition. *Trends Plant Sci.,* **14**, 200–205.

Veisz, O., Galiba, G., Sutka, J. (1996): Effect of abscisic acid on the cold hardiness of wheat seedlings. *J. Plant Physiol.,* **149**, 439–443.

Vranová, E., Inzé, D., van Breusegem, F. (2002): Signal transduction during oxidative stress. *J. Exp. Bot.,* **53**, 1227–1236.

Wang, D. H., Li, X. X., Su, Z. K., Ren, H. X. (2009): The role of salicylic acid in response of two rice cultivars to chilling stress. *Biol. Plant.,* **53**, 545–552.

Xin, Z., Li, P. H. (1992): Abscisic acid-induced chilling tolerance in maize suspension-cultured cells. *Plant Physiol.,* **99**, 707–711.

Xin, Z., Li, P. H. (1993): Relationship between proline and abscisic acid in the induction of chilling tolerance in maize suspension-cultured cells. *Plant Physiol.,* **103**, 607–613.

Yalpani, N., Enyedi, A. J., León, J., Raskin, I. (1994): Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogen-related proteins and virus resistance in tobacco. *Planta,* **193**, 372–376.

Yan, S. P., Zhang, Q. Y., Tang, Z. C., Su, W. A., Sun, W. N. (2006): Comparative proteomic analysis provides new insights into chilling stress responses in rice. *Mol. Cell. Proteomics,* **5**, 484–496.

Yang, S. H., Wang, L. J., Li, S. H. (2007): Ultraviolet-B irradiation-induced freezing tolerance in relation to antioxidant system in winter wheat (*Triticum aestivum* L.) leaves. *Env. Exp. Bot.,* **60**, 300–307.

Yang, T., Poovaiah, B. W. (2002): Hydrogen peroxide homeostasis: Activation of plant catalase by calcium/calmodulin. *Proc. Nat. Acad. Sci. USA,* **99**, 4097–4102.

Yeh, S., Moffatt, B. A., Griffith, M., Xiong, F., Yang, D. S. C., Wiseman, S. B., Sarhan, F., Danyluk, J., Xue, Y. Q., Hew, C. L., Doherty-Kirby, A., Lajoie, G. (2000): Chitinase genes responsive to cold encode antifreeze proteins in winter cereals. *Plant Physiol.,* **124**, 1251–1263.

Yu, C. W., Murphy, T. M., Lin, C. H. (2003): Hydrogen peroxide-induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. *Funct. Plant Biol.,* **30**, 955–963.

Zhang, J., Xu, Y., Huan, Q., Chong, K. (2009): Deep sequencing of Brachypodium small RNAs at the global genome level identifies microRNAs involved in cold stress response. *BMC Genomics,* **10**, 449.

Zheng, Y. Y., Hu, J., Xu, S. C., Guan, Y. J., Wang, X. J. (2009): Relationship between polyamine changes in embryos of maize and seed imbibitional chilling tolerance. *Seed Sci. Technol.,* **37**, 59–69.

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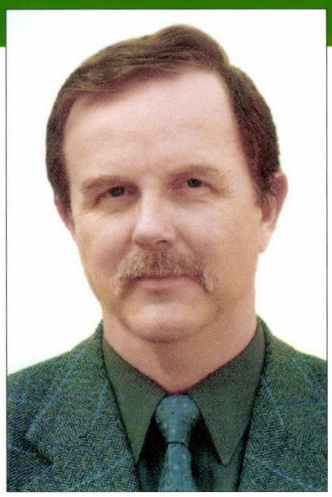

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CONTENTS

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LONG-TERM EFFECT OF CROP PRODUCTION FACTORS ON THE YIELD AND YIELD STABILITY OF MAIZE (*ZEA MAYS* L.) IN DIFFERENT YEARS

Z. BERZSENYI, T. ÁRENDÁS, P. BÓNIS, G. MICSKEI and E. SUGÁR

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The effects of five crop production factors (tillage, fertilisation, plant density, variety, weed control) on the yield and yield stability of maize were examined in Martonvásár (HU) in a polyfactorial experiment and in separate long-term experiments on the effects of N fertilisation, sowing date and plant density. In the polyfactorial experiment the five crop production factors contributed to the increase in maize yield in the following ratios (%): fertilisation 30.6, variety 32.6, plant density 20.2, weed control 14.2, soil cultivation 2.4. In the N fertilisation, sowing date and plant density experiments the effects of the treatments on the maize yield were examined separately for dry and wet years.

Averaged over 40 years, the yields in the long-term N fertilisation experiment were 2.422 t ha^{-1} lower in the dry years than in the wet years (5.170 vs. 7.592 t ha^{-1}). The optimum N rate was 160 kg ha^{-1}. In the sowing date experiment the yield was 2.533 t ha^{-1} lower in the dry years than in the wet years (6.54 vs. 9.093 t ha^{-1}), averaged over 19 years. In dry years the yield was highest for the early and optimum sowing dates, and in wet years for the optimum sowing date. Sowing at dates other than the optimum caused reductions in N fertiliser efficiency. Averaged over 22 years, the optimum plant density was 80,000 plants ha^{-1} in wet years and 50,000 plants ha^{-1} in dry years. The yield was most stable at a plant density of 60,000 plants ha^{-1}. The clarification of year effects is particularly important in relation to the possible effects of climate change.

Key words: maize, long-term experiment, stability analysis, sowing date, N fertilisation, plant density responses

Introduction

In recent years there has been increasing interest in long-term experiments all over the world, as it is only such experiments that provide satisfactory indicators (yield trends, parameters characteristic of the agro-ecosystem) of the sustainability of production and the effect of climate change. The long-term ex-

periments set up in Martonvásár by Béla Győrffy between 1959 and 1961 are now over 50 years old and represent classical long-term experiments. The sustainability of crop production technologies, best indicated by yield stability, is studied in long-term experiments (Árendás et al., 2010; Berzsenyi, 2010).

The improvement in maize production realized in the fields is the result of the combined effects of genetic, ecophysiological and technological changes superimposed on short- and long-term climatological variations. The simple partitioning of yield improvement into a genetic and an agronomic component assumes the lack of interaction between the two components. Although the increase has been the result of both genetic and agronomic-management improvements, most of this improvement is the result of the genotype × management interaction (Tollenaar and Lee, 2002; Duvick et al., 2004).

Maize must be properly managed (selection of appropriate hybrids, planting dates and plant populations) to tolerate the low precipitation and high temperatures that limit yields in dry regions (Norwood, 2001), particularly if they occur during the flowering period. Most growers agree that dryland maize should be planted early so that it can be pollinated before high midsummer temperatures and drought stress occur. Planting maize before or after the optimum date was found to result in reduced leaf area index, leaf area duration, total dry matter production and grain yield. Other research (Nafziger, 1994) has shown an accelerating decline in yield as the planting date is advanced or delayed from the optimum. Among the technological factors, nitrogen fertilisation is the most important factor in increasing maize yields. Under Hungarian conditions, however, water deficit stress regularly limits plant yields and nutrient utilisation (Árendás et al., 2010; Berzsenyi et al., 2011).

This paper discusses the effect of various crop production factors (fertilisation, soil cultivation, sowing date, genotype, plant density and weed control) on the yield and yield stability of maize in different years, based on data series collected over several decades.

Materials and methods

The soil of the experimental area was a humous loam of the chernozem type with forest residues, slightly acidic in the ploughed layer, with poor supplies of available phosphorus and good supplies of potassium.

Long-term polyfactorial experiment

In a long-term experiment set up in 1960, the effect of five crop production factors in increasing maize yields was studied in seven treatments. The factors studied were soil cultivation, fertilisation, plant density, variety and weed control. All the factors had a favourable and an unfavourable level (Table 1). In treatment 1 all the factors were at the unfavourable level and in treatment 2 all were favourable. In treatments 3–7 one of the crop production factors was unfavourable, while all the others were favourable. The unfavourable factors were tillage in treatment 3, fertilisation in treatment 4, plant density in treatment 5, variety in treatment 6 and weed control in treatment 7.

Table 1
Unfavourable and favourable levels of each factor in the polyfactorial experiment

Factor	Unfavourable level	Favourable level
Tillage	Shallow ploughing to a depth of 12–14 cm	Deep ploughing to a depth of 24–28 cm
Fertilisation	No fertilisation	60 t ha^{-1} farmyard manure every 4 years; $N_{140}P_{60}K_{60}$ annually
Plant density	35,000 plants ha^{-1}	70,000 plants ha^{-1}
Variety	Open-pollinated: *Aranyözön* and *Mindszentpusztai Sárga*	Hybrid
Weed control	Late thinning and two delayed hoeings	Complete freedom from weeds; timely thinning

Yield data recorded over 42 years (1960–2001) were evaluated using analysis of variance, regression analysis and stability analysis.

Long-term nitrogen (N) fertilisation experiment

The N fertiliser responses of the maize hybrids were examined in a long-term maize monoculture experiment set up in a split-plot design with four replications in 1961. The N fertiliser rates were as follows (kg ha^{-1}): 0, 80, 160 and 240. The P and K fertiliser rates were the same in all treatments (160 kg ha^{-1}). Since 1970 the N fertiliser response of 10–12 maize hybrids has been investigated each year. In order to analyse the effect of the year on N fertilisation, the 40 years examined between 1970 and 2009 were divided into dry (14) and wet (26) years, based on the quantity of rainfall during the vegetation period (April–September). The yield data were evaluated by analysis of variance.

Long-term sowing date experiment

The effect of sowing date, N fertilisation and genotype on the grain yield of maize was studied between 1991 and 2009 in a long-term N fertilisation experiment set up in 1980. In the three-factor, split-split-plot experiment the N fertiliser treatments represented the main plots, with the sowing date in the sub-plots and the maize hybrid in the sub-sub-plots. The N treatments were as follows: 0, 60, 120, 180 and 240 kg ha^{-1}, while all the treatments received 120 kg ha^{-1} each of P and K. Sowing took place at four dates: 10 days prior to the optimum date (early, S_1), at the optimum date (around April 24, optimum, S_2), ten days after the optimum date (late, S_3) and 20 days after the optimum (very late, S_4). Each year four commercial hybrids were examined, chosen to represent different maturity groups (H1–H4).

Plant density experiment

The effect of plant density on the grain yield of maize was studied in a strip-plot experiment with nine plant densities ranging from 20 to 100 thousand plants per hectare in increments of 10,000 plants ha^{-1}. The effect of year and plant density on the yield and yield stability of maize is presented for an annual average of 20–45 hybrids on the basis of data for 1981–2002.

Analysis of variance

Analysis of variance (ANOVA) for the relevant experimental design was first applied to determine the effects of treatments on the yield in each year. In the second step, the general analysis of variance model (Payne et al., 2010) was used to evaluate the main effects and interactions of the treatments, taking the years into account.

Stability analysis

The stability of the treatments was examined using the single-variable (regression analysis) and multiple-variable (AMMI model) methods of stability analysis. In the regression method of stability analysis the regression between the experimental treatment and the environmental index was calculated. The environmental index is the mean of all the treatments in a given environment (year). Linear regression analysis was carried out according to the methods of Finlay and Wilkinson (1963). A regression coefficient of $b < 1.0$ indicated better adaptation to unfavourable environments, while a value of $b > 1.0$ was characteristic of treatments that showed better adaptation to favourable environments.

The AMMI (Additive Main Effect and Multiplicative Interaction) model is a combination of analysis of variance and principal component analysis (PCA). In the first part of AMMI analysis, ANOVA is carried out to divide the total variation into three orthogonal sources: genotype (G), environment (E) and genotype × environment interaction (G × E). In the second step PCA is applied to dissect the G × E interaction into several orthogonal PCA variables. A biplot is constructed, with the main effect means on the X axis and the PCA I values on the Y axis. The greater the value of PCA I (whether positive or negative) the greater the contribution of the treatment (or environment) to the interaction, i.e. the smaller the yield stability (Crossa, 1990).

Results and discussion

Effect of crop production factors on the yield and yield stability of maize

Time trends in productivity provide strong indicators of the likely long-term sustainability of a crop production system. Further, a comparison of the trends measured for different systems in the same trial may be expected to show which systems are safer and perhaps also suggest which specific factors are responsible for any differences (Jones and Singh, 1999). The time trends for the yields in various production factor combinations in the long-term polyfactorial experiment are shown in Figure 1. The greatest difference in mean yield response was observed between the treatments in which all the factors were unfavourable or favourable (2.09 and 8.59 t ha^{-1}). When fertilisation or the variety was the unfavourable factor, there was a considerable reduction of >3.0 t ha^{-1} in yield compared to the favourable level (mean yield response 5.21 and 4.98 t ha^{-1}, respectively). When plant density or weed control was unfavourable, the yield loss was 2.2 or 1.6 t ha^{-1}, respectively (mean yield response 6.36 and 7.01 t ha^{-1}, respectively). Compared with the other production factors the unfavourable level of weed control exhibited the largest environment dependence. Figure 1 shows that the unfavourable depth of soil tillage only had a slight effect on the maize yield (mean yield response: 8.21 t ha^{-1}). It is clear from Figure 1 that fertilisation and

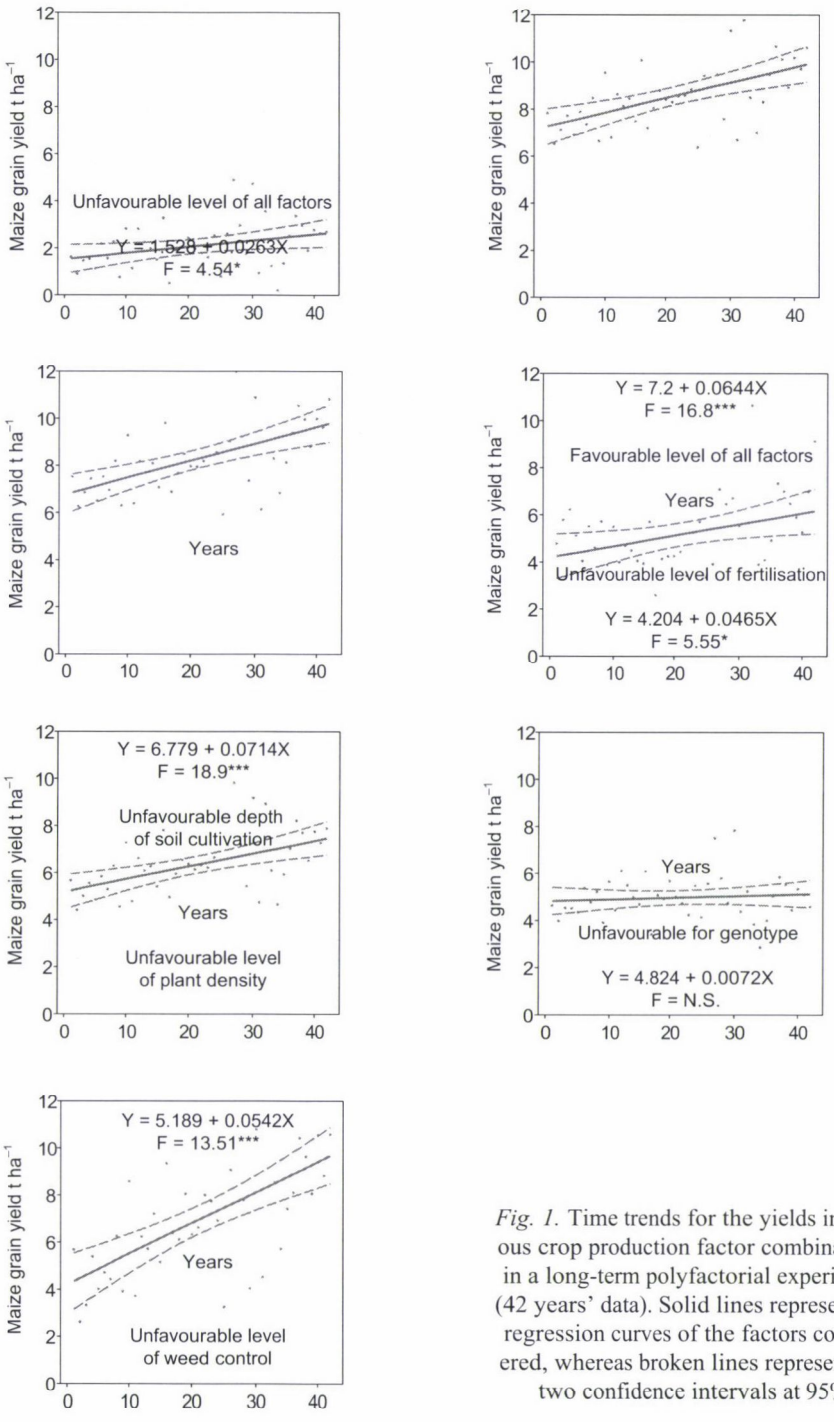

Fig. 1. Time trends for the yields in various crop production factor combinations in a long-term polyfactorial experiment (42 years' data). Solid lines represent the regression curves of the factors considered, whereas broken lines represent the two confidence intervals at 95%

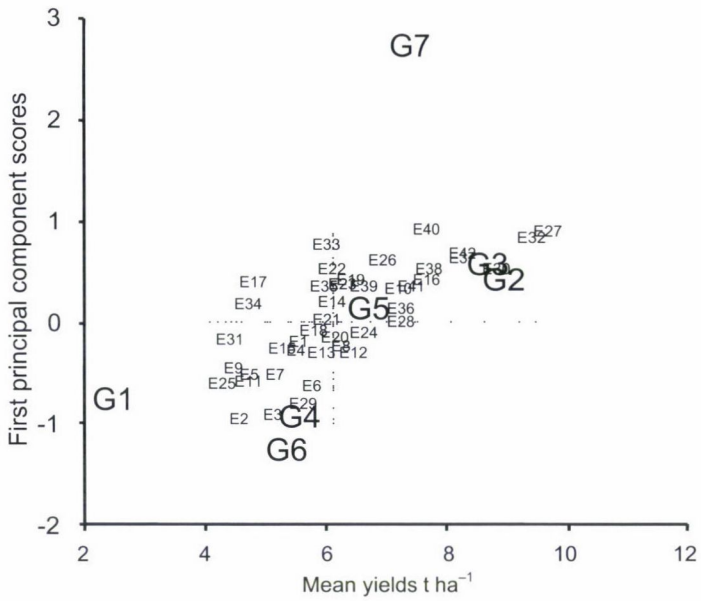

Fig. 2. Plot of the mean yields and first principal component scores of seven crop production factors (G1–G7) in 42 environments (E1–E42)

genotype made the greatest contribution to sustainability, followed by the plant density, weed control and the depth of tillage. The crop production factors contributed to the increase in maize yield in the following ratios (%): fertilisation 30.6, variety 32.6, plant density 20.2, weed control 14.2, soil cultivation 2.4 (Berzsenyi and Dang, 2008a).

The results of the AMMI analysis are illustrated in Figure 2, with the yield average on the X axis and the value of the first principal component on the Y axis, for seven treatments (G1–G7) in 42 environments (E1–E42). It can be seen that Treatments 7, 6, 1 and 4 made the greatest contribution to the interaction, while Treatments 2, 3 and 5 had the greatest yield stability. Evaluating the mean performance and yield stability together, the best treatment was Treatment 2, which combined the favourable levels of all the production factors, followed by Treatment 3, where tillage was at the unfavourable level and the other factors were favourable.

Long-term nitrogen (N) fertilisation experiment

The effect of N fertilisation and the year on the maize grain yield was investigated for 14 dry and 26 wet years between 1970 and 2009. The rainfall in the growing season averaged 222 mm in the dry years and 348 mm in the wet years.

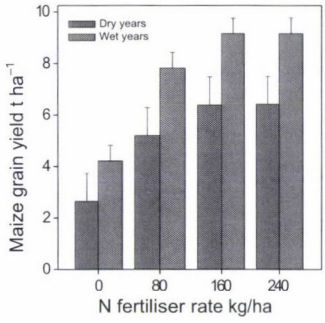

Fig. 3a. Effect of N fertilisation on maize grain yield in dry (14) and wet (26) years (1970–2009)

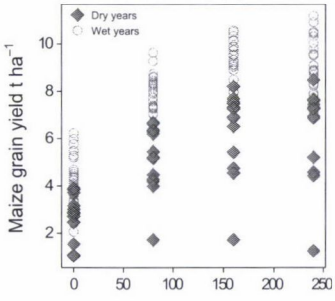

Fig. 3b. Scatter plot of the yearly maize yield response in dry and wet years

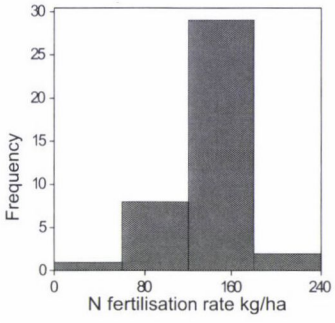

Fig. 3c. Histogram of optimum N rates from ANOVA

When comparing the wet and dry years it was found that in wet years the yield increment achieved in each N treatment was as follows (t ha^{-1}): N_0: 1.567, N_{80}: 2.616, N_{160}: 2.764, N_{240}: 2.74 (Fig. 3a). There were considerable fluctuations over the years in the N-fertiliser responses of the maize hybrids (Fig. 3b). Analysis of variance on the 40-year data series showed that the significantly highest yield was most frequently achieved with the N_{160} dose (Fig. 3c). *Ex ante* data that form the basis of farming decisions can only be obtained from long-term experiments.

Long-term sowing date experiment

The effect of N fertilisation, sowing date and hybrid on the maize grain yield in 12 dry and 7 wet years is illustrated in Figure 4. Averaged over the treatments, the yield was 2.553 t ha^{-1} greater in wet years than in dry years (9.093 vs 6.540 t ha^{-1}). In dry years the yield was highest in the early and optimum sowing date treatments (7.083 and 6.880 t ha^{-1}), with a significant decrease when sowing

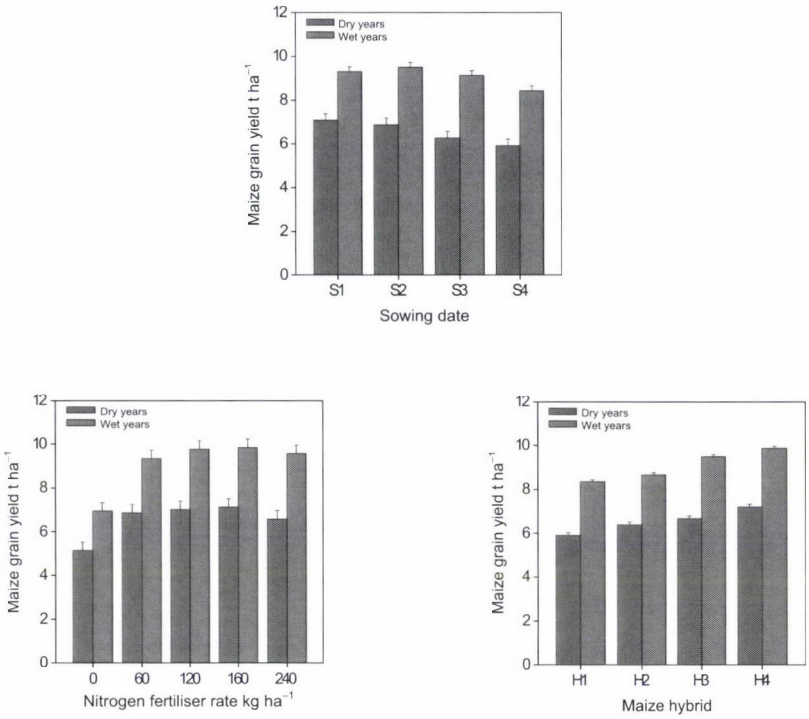

Fig. 4. Effect of N fertilisation, sowing date and hybrid on the grain yield of maize in dry (7) and wet (12) years

took place 10 or 20 days later (6.273 and 5.925 t ha^{-1}). In dry years there was no significant difference in the maize yields achieved at N rates of 60, 120 and 180 kg ha^{-1}, while a significant reduction was recorded at the 240 kg ha^{-1} rate. In wet years the yield was significantly higher for the optimum sowing date (t ha^{-1}): early: 9.312, optimum: 9.5, late: 9.131, very late: 8.431. The optimum N rate was 120 kg ha^{-1} in wet years; at higher rates there was no significant change in the yield. In both types of years sowing later than the optimum date led to a reduction in N fertiliser efficiency, which was more severe in the dry years. Of the hybrids tested, the yields of H1 and H2 were similar, as were those of H3 and H4. In both dry and wet years hybrids with long vegetation periods (H3, H4) produced significantly greater yields. According to earlier research (Berzsenyi and Dang, 2008b), the greatest yield stability was recorded for sowing at the optimum date or 10 days later and for N fertiliser rates of 60 or 120 kg ha^{-1}.

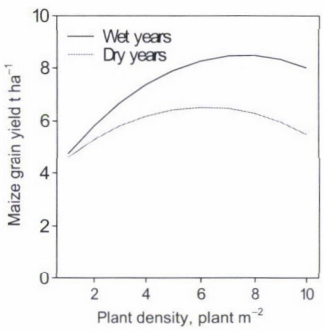

Fig. 5a. Effect of plant density on the maize grain yield in different years (1981–2002)

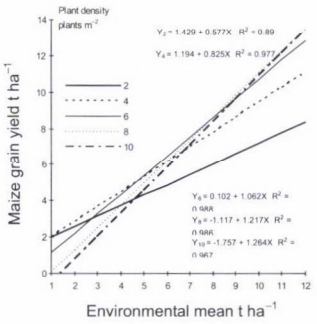

Fig. 5b. Yield stability of maize hybrids at different plant densities in the period 1981–2002 (average of 20–45 hybrids yearly)

Plant density experiment

In the plant density experiment, the maximum grain yield, averaged over the wet years, was 8.21 t ha^{-1}, with an optimum plant density of 80,000 plants ha^{-1}. By contrast, averaged over the dry years the maximum grain yield was 6.65 t ha^{-1} and the optimum plant density was only 50,000 plants ha^{-1}. In dry years above-optimum plant density resulted in considerable yield losses (as indicated by the increasing distance between the two curves; Fig. 5a). Under the given experimental conditions, the yield was most stable at a plant density of 60,000 plants ha^{-1}. When the environmental mean was less than 4.6 t ha^{-1} a plant density of 40,000 plants ha^{-1} had greater stability, while a plant density of 80,000 plants ha^{-1} could be expected to be more stable at environmental means of over 7.9 t ha^{-1} (Fig. 5b).

References

Árendás, T., Bónis, P., Csathó, P., Molnár, D., Berzsenyi, Z. (2010): Fertiliser responses of maize and winter wheat as a function of year and forecrop. *Acta Agron. Hung.*, **58** (Suppl.), 109–114.

Berzsenyi, Z. (2010): Significance of 50-year-old long-term experiments in Martonvásár in improving crop production. *Acta Agron. Hung.*, **58** (Suppl.), 23–34.

Berzsenyi, Z., Dang, Q. L. (2008a): Effect of various crop production factors on the yield and yield stability of maize in a long-term experiment. *Cereal Res. Commun.*, **36**, 167–176.

Berzsenyi, Z., Dang, Q. L. (2008b): Effect of sowing date and N fertilisation on the yield and yield stability of maize (*Zea mays* L.) hybrids in a long-term experiment. *Acta Agron. Hung.*, **56,** 247–267.

Berzsenyi, Z., Árendás, T., Bónis, P., Micskei, G. (2011): Long-term effect of crop production factors on maize productivity in different years. In: Veisz, O. (ed.): *Climate Change: Challenges and Opportunities in Agriculture*. Agrisafe Final Conference, March 21–23, 2011, Budapest, pp. 374–377.

Crossa, J. (1990): Statistical analyses of multilocation trials. *Adv. Agron.,* **44,** 55–85.
Duvick, D. N., Smith, J. S. C., Cooper, M. (2004): Changes in performance, parentage, and genetic diversity of successful corn hybrids, 1930–2000. pp. 65–97. In: Smith, C. W. (ed.), *Corn: Origin, History, Technology, and Production*. John Wiley & Sons, Inc., New York.
Finlay, K. W., Wilkinson, G. N. (1963): The analysis of adaptation in a plant breeding programme. *Aust. J. Agric. Res.,* **14**, 742–754.
Jones, M. J., Singh, M. (1999): Time trends in crop yields in long-term trials. *Expl. Agric.,* **36**, 165–179.
Nafziger, E. D. (1994): Corn planting and plant population. *J. Prod. Agric.,* **7**, 59–62.
Norwood, C. A. (2001): Dryland corn in Western Kansas: Effects of hybrid maturity, planting date, and plant population. *Agron. J.,* **93**, 540–547.
Payne, R., Welham, S., Harding, S. (2010): *A Guide to REML in GenStat*. VSN International, Hertfordshire.
Tollenaar, M., Lee, E. A. (2002): Yield potential, yield stability and stress tolerance in maize. *Field Crops Res.,* **75**, 161–169.

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EFFECT OF ABIOTIC STRESS FACTORS ON THE CHLOROPHYLL CONTENT OF INBRED MAIZE LINES

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Inbred maize lines were treated with normal and double rates of post-emergence herbicides in a small-plot field experiment in one dry and one wet year. The chlorophyll *a* + *b* content of symptom-free ear-leaves was determined using a spectrophotometer after 50% silking in order to determine whether various rates of post-emergence herbicides had any effect on the chlorophyll content at flowering and how this was influenced by the type of year. The chlorophyll *a* + *b* content of the inbred lines was greatly dependent on the year, with values twice as high in the wet year as in the dry year. Treatment with tembotrione + isoxadifen-ethyl had no effect on the chlorophyll content in either year. Both rates of mesotrione + terbutylazine reduced the chlorophyll *a* + *b* content of one stress-sensitive inbred line in the dry year, but not in the wet year. In the wet year bentazone + dicamba increased the chlorophyll content, but only for one line was this effect significant irrespective of the dose. In the dry year the double dose caused a significant increase in this genotype, but the chlorophyll contents of the other lines did not differ significantly from the control.

Key words: post-emergence herbicide, inbred maize line, chlorophyll content, year effect

Introduction

Herbicide application is essential if intensive maize production is to be successful. As some herbicides may damage certain maize genotypes under unfavourable conditions, it is advisable to check whether their application is safe before they are widely used. This is particularly important for the inbred lines sown for seed production, as these are well known to be more sensitive to environmental effects than the hybrids developed from them (Green and Ulrich, 1993; Berzsenyi et al., 1994; Green, 1998; Bónis et al., 2004).

The damage caused by herbicides may range from alterations imperceptible to the naked eye to clearly visible symptoms, including plant mortality. In the case of non-lethal damage, the plants generally exhibit gradual regeneration over the vegetation period, and the symptoms become masked or may even completely disappear. Biotic and abiotic stress factors may cause pathological changes to the photosynthetic apparatus of higher plants (Almási et al., 2005). Some herbicide active agents exert their effect by inhibiting photosynthetic processes (Kádár, 2010). One characteristic symptom in the majority of herbicides, especially those acting as photosynthetic electron transport inhibitors, is the yellowing or whitening of the leaves (chlorosis), which is the consequence of the irreversible photodestruction of chlorophyll and other pigments (Szigeti and Vágújfalvi, 1983). It was observed by Sunohara et al. (2010) that auxinic herbicides also influence the chlorophyll content of weeds.

The weather extremes occurring in some years may also have a substantial effect on the extent and duration of the abiotic stress induced by herbicides.

Materials and methods

The responses of maize inbred lines to herbicides were investigated in a small-plot field experiment in Martonvásár in two years with very diverse weather conditions (Table 1). In 2009,

Table 1
Rainfall and temperature conditions during the vegetation period of maize (Martonvásár)

Month	10-day period	Rainfall (mm)			Mean temperature (°C)			Very hot days (t_{max} > 30°C)		
		2009	2010	30-year mean	2009	2010	30-year mean	2009	2010	1999–2008
Apr.	1	0.1	13.2	12	13.5	9.4	10.4	0	0	0
	2	2.0	27.5	13	14.0	10.4	10.8	0	0	0
	3	0	0.1	18	14.4	13.5	12.6	0	0	0
May	1	0.6	8.4	18	14.8	16.7	14.8	0	0	1
	2	0.7	102.0	16	17.2	12.5	17.0	1	0	1
	3	11.3	65.7	22	16.8	17.3	17.3	0	0	2
June	1	7.4	30.7	26	17.7	18.5	19.1	0	2	2
	2	12.5	47.0	22	18.9	20.9	19.5	2	3	3
	3	49.9	46.1	25	18.8	17.8	20.6	1	2	4
July	1	4.1	1.0	18	20.8	21.6	21.0	5	4	4
	2	17.6	0.0	16	22.0	25.1	22.0	5	8	5
	3	1.1	35.6	19	22.6	21.0	21.5	8	3	5
Aug.	1	23.7	53.8	18	23.0	20.2	21.6	3	2	5
	2	5.5	51.0	15	21.0	21.6	21.0	3	4	4
	3	13.6	44.1	13	20.5	18.8	19.6	4	1	3
Sep.	1	4.1	48.8	10	20.8	13.8	18.8	2	0	1
	2	17.6	71.6	14	22.0	15.5	16.4	0	0	0
	3	1.1	34.5	17	22.6	13.3	14.6	0	0	0
Σ or mean		159.5	681.1	312	19.0	17.1	17.7	34	29	40

which was hotter and drier than usual, the rainfall in the growing season (173 mm) was only a little more than half the 30-year mean (312 mm), while the mean temperature was 1.3°C higher than the 30-year mean (19°C). In 2010, however, the weather was cool (17.1°C) and wet (681 mm).

The experiment was set up in a three-factor split-plot design with two replications in 2009 and four replications in 2010, with an untreated control plot for each treatment. The active agents in the herbicides were as follows: mesotrione + terbutylazine, bentazone + dicamba, tembotrione + isoxadifen-ethyl. The treatments are presented in Table 2.

Table 2
Treatments in the herbicide sensitivity experiment

Treatment	Dose (l a.i. ha^{-1})	
	Normal	Double
1. Control	–	–
2. Mesotrione + Terbutylazine	140 + 660	660 + 1320
3. Bentazone + Dicamba	960 + 270	1920 + 540
4. Tembotrione + Isoxadifen-ethyl	99 + 49.5	198 + 99

The herbicides were applied post-emergence in the 5–7-leaf stage of three Martonvásár maize inbred lines (Line 1, Line 2, Line 3) using the maximum recommended dose and double this dose. Samples were taken after 50% silking from symptom-free ear-leaves. The chlorophyll $a + b$ content was recorded with a Hitachi U-1500 spectrophotometer using the method of Arnon (1949) in order to determine whether various doses of post-emergence herbicides had any detectable effect on the chlorophyll content during flowering. The data were evaluated with two- and three-factor analysis of variance using the M-STAT C program.

Results and discussion

Three-factor analysis of variance (Table 3) revealed that the chlorophyll content of the maize leaves was affected to the greatest extent by the genotype in both years and this effect was significant at the P = 0.1% level. The herbicides had no significant effect in the dry year, while in the wet year they significantly influenced the chlorophyll content of maize leaves at the P = 0.1% level. The results showed that the herbicide dose had the smallest effect, which was not significant in either year, averaged over lines and active ingredients.

The effect of year and herbicide treatment on the chlorophyll content of inbred lines is illustrated in Figure 1 as a function of the application rate. The decisive effect of the year is shown by the fact that the chlorophyll $a + b$ content of the ear-leaf was more than twice as high in 2010 than in the dry year. In 2009 a rise in the mesotrione + terbutylazine dose caused a significant reduction in the chlorophyll content compared with the control, averaged over the genotypes. In the wet year a reduction was only recorded for the double dose, but this was not significant. While treatment with tembotrione + antidote caused no significant change in the chlorophyll $a + b$ content in either year, the bentazone + dicamba herbicide combination resulted in a significant increase in the chlorophyll $a + b$ content in 2010, averaged over the lines (Fig. 1).

Table 3
Analysis of variance for the three-factor experiment

Factor	2009		2010	
	df	MQ	df	MQ
Replication	1	13654.92	3	3792.48
Herbicide (A)	2	11443.35	2	668516.99***
Error (a)	2	17694.30	6	47119.97
Dose (B)	2	12912.97	2	60893.56
A×B	4	25750.75	4	166017.30
Error (b)	6	21748.37	18	56912.47
Line (C)	2	306545.28***	2	5321487.47***
A×C	4	33192.66*	4	822461.21***
B×C	4	13806.75	4	20218.46
A×B×C	8	aug.96	8	14910.62
Error (c)	18	8826.90	54	52955.58

* Significant at the P = 5% level
*** Significant at the P = 0.1% level

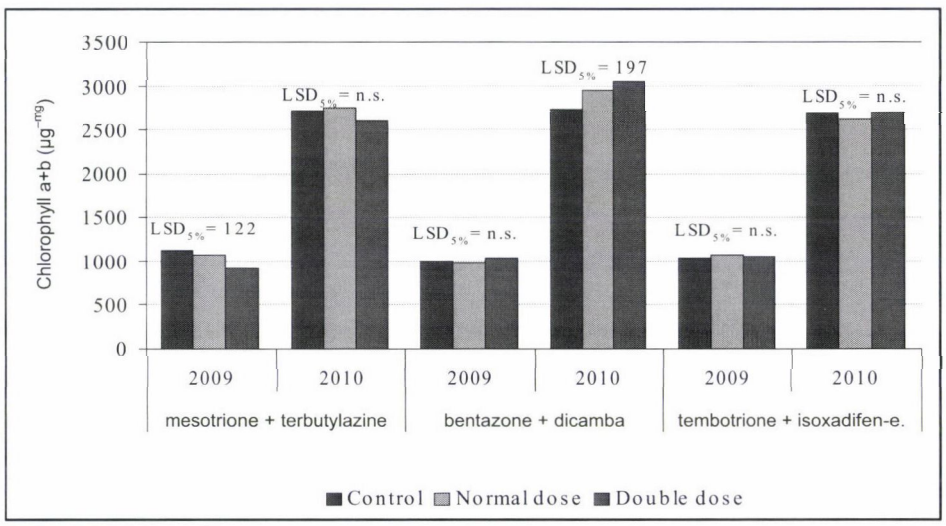

Fig. 1. Effect of herbicide treatments on the chlorophyll content in different years, averaged over the genotypes (Martonvásár)

In the dry year the chlorophyll *a* + *b* content of Line 1 was reduced by more than 15% by the normal dose of mesotrione + terbutylazine and by more than 30% by the double dose (Fig. 2). In the wet year neither dose caused a significant change in the chlorophyll content compared with the control. The tembotrione + isoxadifen-ethyl treatment had practically no influence on the chlorophyll content of any of the inbred lines in either year (Figs. 2–4).

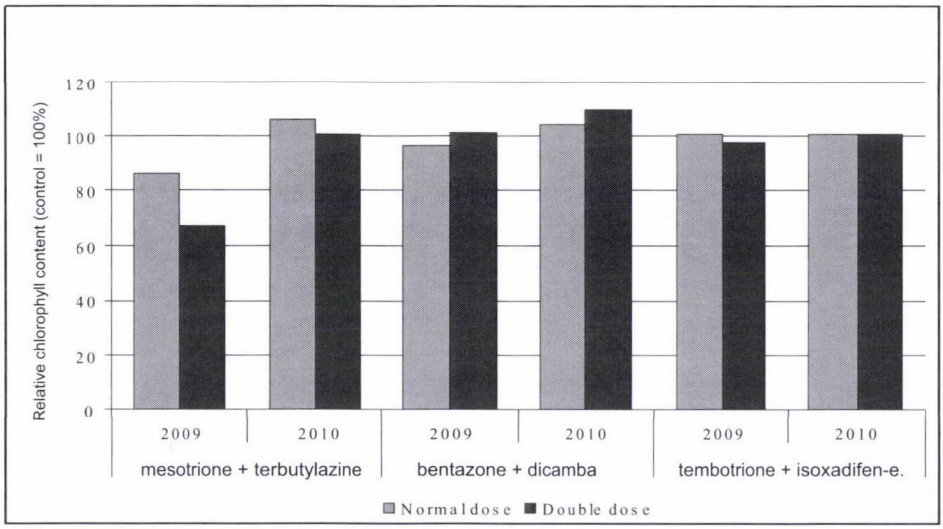

Fig. 2. Changes in the relative chlorophyll *a* + *b* content of Inbred line 1 compared with the control, as a function of herbicides and doses in different years

In the wet year the bentazone + dicamba treatment increased the chlorophyll content in all three lines, but the rise was only significant, irrespective of the dose, in Line 3. In the dry year a significant increase was only observed for the same genotype in the case of the double dose, while the chlorophyll contents of the other lines did not differ significantly from the control.

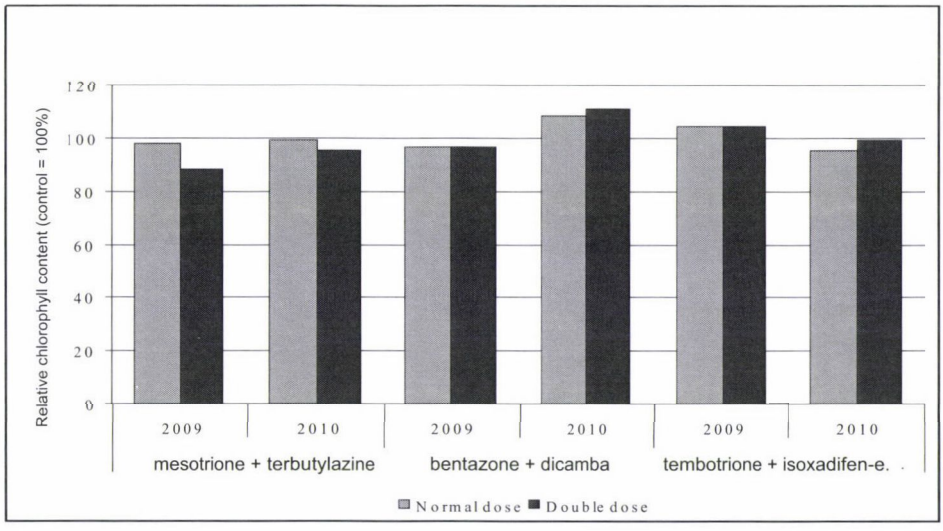

Fig. 3. Changes in the relative chlorophyll *a* + *b* content of Inbred line 2 compared with the control, as a function of herbicides and doses in different years

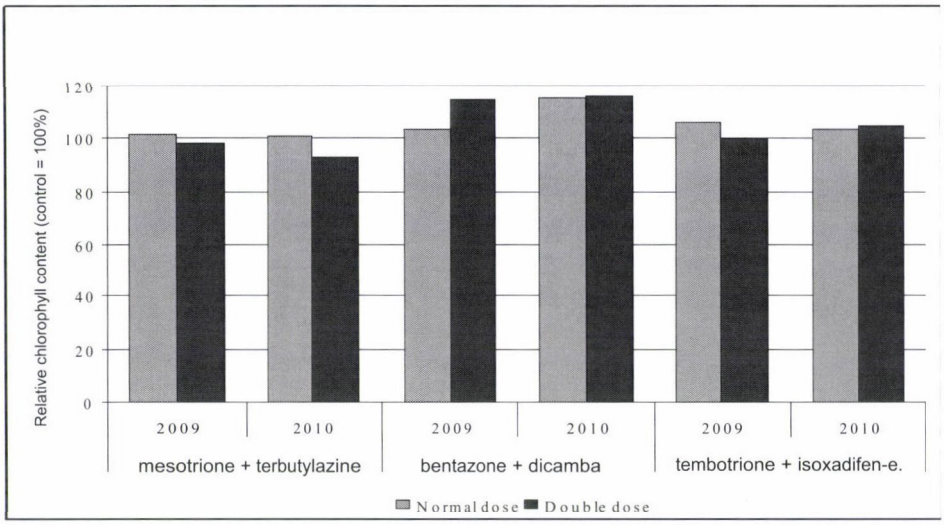

Fig. 4. Changes in the relative chlorophyll *a* + *b* content of Inbred line 3 compared with the control, as a function of herbicides and doses in different years

Conclusions

The year was found to have the greatest influence on the chlorophyll content of herbicide-treated maize lines at flowering. In the wet year the chlorophyll *a* + *b* content was more than twice as high as in the dry year.

In both years the line had a significant effect on the chlorophyll content, while the herbicides only influenced the chlorophyll *a* + *b* content of the ear-leaf in the wet year. The dose had no significant effect in either year.

The herbicides tested induced diverse responses from the inbred lines. Differences in the chlorophyll content could be detected between the lines as a function of year and active ingredient, suggesting that this method could be used in later stages of development to identify maize genotypes that, though they exhibit no visible symptoms, have not succeeded in completely neutralising the damaging effects of post-emergence herbicides.

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References

Almási, A., Sárvári, É., Bóka, K., Lózsa, R., Sági, Z., Gáborjányi, R. (2005): A klorofill-protein komplex változásai tobamovírusokkal fertőzött, eltérő rezisztencia-típusú paprikanövényekben (Changes in chlorophyll-protein complexes in tobamovirus infected pepper varieties with different levels of resistance.) *Növényvédelem,* **41**, 349–354.

Arnon, D. I. (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Phys.,* **24**, 1–15.

Berzsenyi, Z., Bónis, P., Árendás, T., Berényi, G. (1994): Comparative investigations on the efficacy and selectivity of different herbicides in maize. *Z. PflKrankh. PflSchutz, Sonderh.,* **14**, 457–466.

Bónis, P., Árendás, T., Berzsenyi, Z., Marton, L. C. (2004): Herbicide tolerance studies on maize inbred lines. *Z. PflKrankh. PflSchutz, Sonderh.,* **19**, 901–907.

Green, J. M. (1998): Differential tolerance of corn *(Zea mays)* inbreds to four sulfonylurea herbicides and bentazon. *Weed Technol.,* **12**, 474–477.

Green, J. M., Ulrich, J. F. (1993): Response of corn *(Zea mays)* inbreds and hybrids to sulfonylurea herbicides. *Weed Sci.,* **41**, 508–516.

Kádár, A. (ed.) (2010): *Vegyszeres gyomirtás és termésszabályozás. (Chemical weed control and yield regulation.)* Private publication, 382 pp.

Sunohara, Y., Shirai, S., Wongkantrakorn, N., Matsumoto, H. (2010): Sensitivity and physiological responses of *Eleusine indica* and *Digitaria adscendens* to herbicide quinclorac and 2,4-D. *Environ. Exp. Bot.,* **68**, 157–164.

Szigeti, Z., Vágújfalvi, D. (1983): Klorofill fotooxidáció serkentése herbicidekkel és kivédése reduktánsokkal. (Enhancement of chlorophyll photooxidation with herbicides and protection against it with reductants.) *Bot. Közl.,* **70**, 159–164.

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IN VITRO SELECTION OF MICROSPORES FOR RESISTANCE TO OXIDATIVE STRESS RESULTED IN CHILLING TOLERANCE IN DOUBLED HAPLOID MAIZE LINES

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The chilling tolerance of doubled haploid (DH) maize plants selected and regenerated from microspores exposed to prooxidants, paraquat or *tert*-butyl hydroperoxide was determined by monitoring cold-induced changes in the photosynthetic electron transport, CO_2 assimilation processes and chlorophyll breakdown in young leaves after cold treatment (8°C for 5 days). The results were compared to those of the non-selected DH line and the original hybrid plants. Chilling stress caused a great reduction in the Fv/Fm, qP and ΔF/Fm' fluorescence parameters, related to the photosynthetic electron transport processes, and in carbon assimilation, and resulted in chlorophyll breakdown. These changes were less extensive in the selected DH plants, which showed elevated antioxidant capacity both at ambient and at low temperature. Among the antioxidant enzymes tested, the activity of GR and GST was induced by chilling stress to the greatest extent. Correlations between cold-induced changes in the photosynthetic apparatus and the antioxidant capacity of the plants suggested that the better protection against oxidative stress induced by the elevated antioxidant capacity of the plants contributed to protecting the photosynthetic apparatus from cold.

Key words: chilling, doubled haploid maize, oxidative stress

Introduction

Maize (*Zea mays* L.) is an agronomically important crop. Due to its tropical or subtropical origin, it is considered as a typically chilling-sensitive plant, which requires a relatively high temperature for optimal growth. However, it is also cultivated in temperate regions, where it is often subject to low temperature stress, especially during the early stages of growth. The optimal temperature for maize is between 20 and 30°C and temperatures below 15°C may induce chilling stress, resulting in retarded development.

Like many other aspects of maize physiology, the photosynthetic apparatus appears to be severely affected by chilling. At low temperature, a decrease in photosynthetic electron transport activity, a reduction in the activity of the enzymes responsible for PEP carboxylation and the Calvin cycle (especially the Rubisco enzyme) and stomatal closure are observed in maize, resulting in low photosynthetic activity (Allen and Ort, 2001).

The limited photochemical utilization of absorbed light energy induces heat dissipation processes, disturbs the redox homeostasis in the cells and increases the risk of producing reactive oxygen species, ROS (Apel and Hirt, 2004; Miller et al., 2008). Damage from exposure to chilling stress may be mediated by the formation of ROS such as superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals, resulting in the oxidative degradation of biomolecules (chlorophylls, lipids, proteins, nucleic acids).

Plants have evolved several mechanisms to prevent or alleviate damage by ROS. These mechanisms include the scavenging of ROS by antioxidant compounds and antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidise (APX), catalase (CAT), glutathione reductase (GR) and glutathione S-transferase (GST) (Apel and Hirt, 2004). Therefore, an improvement in the antioxidant capacity of the plants may enhance their stress tolerance, helping them to cope with short-term low-temperature stress.

At the Agricultural Research Institute of the Hungarian Academy of Sciences, the doubled haploid technology was used to select and regenerate fertile DH maize plants from microspores exposed to ROS progenitors, such as paraquat or *t*-butylhydroperoxide (*t*-BuOOH) (Ambrus et al., 2006). The progenies of the selected DH plants had enhanced tolerance to the oxidative stress induced by the selection agents and elevated antioxidant capacity compared both to the original hybrid plants and to control DH (DH C) plants derived from microspores not exposed to agents producing ROS (Darkó et al., 2009).

In the present work, changes induced by chilling in the photosynthetic electron transport and CO_2 assimilation processes were compared in young, DH maize lines tolerant of oxidative stress, originating from microspores exposed to paraquat or *t*-BuOOH. The role of antioxidant enzymes in protection against chilling stress was also investigated.

Materials and methods

Progenies of DH maize (*Zea mays* L.) plants selected and regenerated from microspores exposed to paraquat or *t*-BuOOH (Ambrus et al., 2006) were used in the experiments. Chilling stress was induced by transferring young 2-leaf plants to 8°C for 5 days. Control plants were kept at day/night temperatures of 22/20°C. The light intensity was 200 μmol m^{-2} s^{-1} in both growth chambers. Three plants of each paraquat- (PqR1–4) and *t*-BuOOH-selected line (BR1–BR4), three hybrid plants (H) and 10 different DH C plants were used for each treatment. The experiments were repeated three times and the mean ± S.D. values are presented.

Photosynthetic electron transport processes were followed by chlorophyll *a* fluorescence measurements (PAM 2000 Chl *a* fluorometer) performed on intact, attached leaves. The initial (Fo)

and maximal (Fm) fluorescence levels were determined after 15 min dark adaptation and the fluorescence quenching parameters were determined at the steady-state level of photosynthesis after illumination at 340 μmol m^{-2} s^{-1} light intensity. The fluorescence parameters, Fv/Fm (relating to the primary charge separation capacity of PSII), ΔF/Fm' (estimating the quantum yield of linear electron flux) and NPQ (non-photochemical quenching, reflecting the heat dissipation of the excitation energy) were calculated according to van Kooten and Snel (1990).

The CO_2 assimilation processes were monitored using an infrared gas analyser (LCA-2, Analytical Development Co., Ltd, Hoddesdon, UK) as described by Darkó et al. (2011). Measurements were carried out at relevant temperatures (8°C or 22°C) on intact, attached leaves after 20 min illumination with saturating light (700 μmol m^{-2} s^{-1}). The net CO_2 assimilation rate (A) and the stomatal conductance (gs) parameters were calculated using the equation of von Caemmerer and Farquhar (1981).

The chilling-induced pigment degradation was monitored by determining the chlorophyll *(a + b)* content of the leaves with a Cary-100 UV-Vis spectrophotometer (Varian, Mulgrave, Australia) in 80% acetone, according to Lichtenthaler (1987).

The role of antioxidant capacity in protecting plants from chilling stress was determined by monitoring the activities of antioxidant enzymes (superoxide dismutase, SOD; ascorbate peroxidase, APX; catalase, CAT; glutathione reductase, GR and glutathione S-transferase, GST) using spectrophotometric methods as described in Darkó et al. (2009).

Results and discussion

In vivo chlorophyll fluorescence measurements are commonly used to study the functioning of the photosynthetic apparatus, the response of plants to environmental stress, including the effects of low temperature on the photosynthetic apparatus, and as a screening method to evaluate the chilling tolerance of maize genotypes (Lee et al., 2002; Janda et al., 2005).

When the hybrid (H), non-selected (DH C) and selected (PQR1–4, BR1–4) DH maize lines were grown at ambient (22/20°C) day/night temperatures, there were no significant differences in the fluorescence induction and quenching parameters, Fv/Fm (related to the primary charge separation capacity of PSII), qP and ΔF/Fm' (estimating the quantum yield of PS II photochemistry and linear electron flux, respectively) and NPQ (reflecting the heat dissipation of excess excitation energy) (Fig. 1, light bars). These results indicated that at ambient temperature the photosynthetic electron transport activity was similar in all the plants.

When the plants were exposed to low temperature stress (8°C for 5 days), the fluorescence quenching parameters (except NPQ) decreased significantly (Fig. 1, dark bars). Decreases in the primary charge separation capacity (indicated by the decline in Fv/Fm) and in the efficiency of linear electron transport (low ΔF/Fm') and increases in the closure of PS II reaction centres (low qP) were especially pronounced in H and DH C plants, but less evident in oxidative stress-tolerant plants derived from microspores exposed to paraquat (PqR1–4) or *t*-BuOOH (BR1–4), suggesting that ROS-tolerant plants had elevated cold tolerance. In addition, as reflected by the NPQ parameters, the excess excitation energy can be eliminated by heat in some ROS-tolerant plants, reducing the risk of ROS forma-

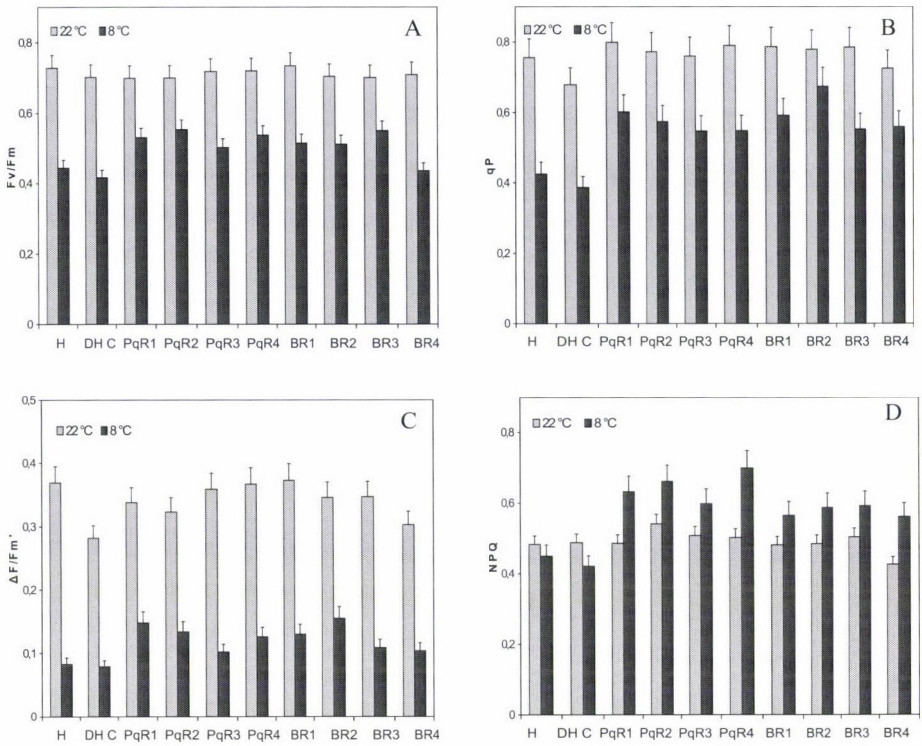

Fig. 1. The optimal, Fv/Fm (A) and effective, ΔF/Fm'(C) quantum yield of PS II, and the photochemical, qP (B) and non-photochemical, NPQ (D) quenching parameters in leaves of different DH maize lines and hybrid plants after cold treatment (at 8°C for 5 days). For control measurements, the plants were kept at 22°C

tion (Fig. 1D). However, the role of heat dissipation in the protection of the photosynthetic apparatus is limited even in these plants.

In maize, as in most C_4 plants, the enzymes responsible for CO_2 fixation have a high temperature optimum (Crafts-Brandner and Salvucci, 2000), so chilling stress greatly inhibits CO_2 assimilation processes (Fryer et al., 1998). Low temperature may also induce stomatal closure (Wilkinson et al., 2001). In the present study, the hybrid plants had higher CO_2 fixation capacity than any of the DH lines at ambient temperature, whereas no significant differences were observed among the DH lines (Fig. 2A). Cold stress resulted in a greatly reduced CO_2 assimilation rate (A), especially in the H and DH C genotypes (Fig. 2A). The gs parameter, reflecting stomatal closure, did not change significantly in any of the plants under chilling stress conditions (Fig. 2B), indicating that the decrease in A was not due to stomatal closure, which limits the CO_2 availability for the Rubisco or PEP carboxylase enzymes. The absence of stomatal closure and the correlation ($r = 0.83$) between the effective quantum yield of PS II (ΔF/Fm') and

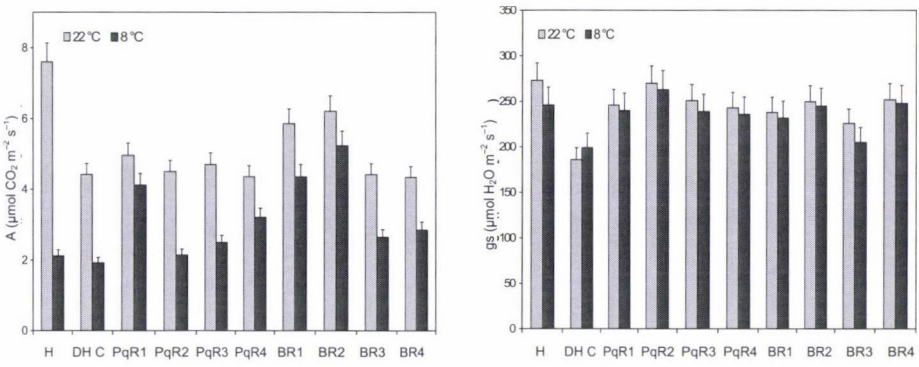

Fig. 2. CO_2 assimilation rate (A) and stomatal conductance (gs) measured on intact leaves of different DH maize lines and hybrid plants after cold treatment (at 8°C for 5 days). For control measurements, the plants were kept at 22°C

A suggested that the cold-induced decrease in the photosynthetic activity of DH maize lines was primarily associated with the inhibition of electron transport processes, leading to NADPH depletion.

After 5 days, the low temperature stress resulted in a decrease in the chlorophyll content of the leaves, especially in the H and DH maize lines (Fig. 3). The chlorophyll bleaching was less pronounced in the oxidative stress-tolerant DH plants (PqR1–4, BR1–4).

The role of antioxidant enzymes in counteracting cold stress was tested by comparing the antioxidant capacity of selected DH plants to that of non-selected DH C and hybrid plants with and without low temperature stress. Antioxidant capacity was calculated by summing the normalized activities of SOD, CAT, APX, GR and GST recorded in maize leaves under the experimental conditions described above for photosynthesis measurements (Fig. 4).

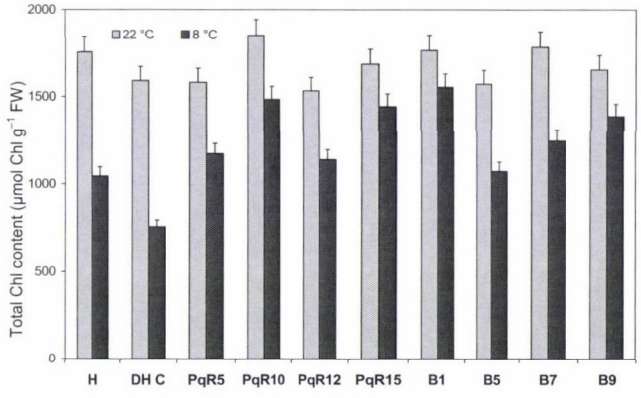

Fig. 3. Total chlorophyll content of leaves in different DH maize lines and hybrid plants after cold treatment (at 8°C for 5 days). For control measurements, the plants were kept at 22°C

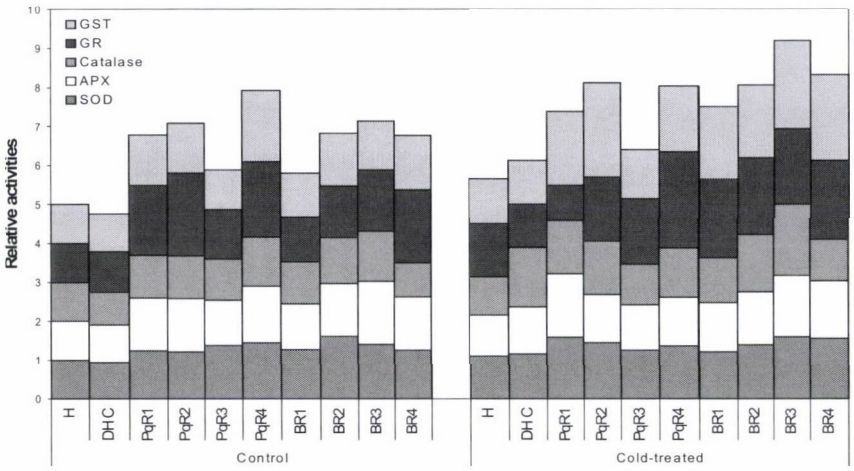

Fig. 4. Relative activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and glutathione S-transferase (GST) in the leaves of different DH maize lines and hybrid plants with (8°C) and without (22°C) chilling stress. The enzyme activities were normalized to those measured in the control leaves of hybrid plants. An activity level of 1 represented 8.4, 3.37, 15.5, 0.289 and 2.22 μM g^{-1} FW min^{-1} activities of SOD, APX, CAT, GR and GST, respectively

In both unstressed and cold-treated leaves, most of these antioxidant enzymes exhibited higher activities in the oxidative-stress tolerant DH lines than in H and DH C plants, indicating the simultaneous up-regulation of the antioxidant enzymes in paraquat- or *t*-BuOOH-selected DH lines (Fig. 4). Cold stress significantly stimulated the activity of GR in most of the plants, and of GST in oxidative stress-tolerant DH lines. Catalase was also induced in some oxidative stress-tolerant DH lines (Fig. 4). Cold stress seemed to have no effect on the SOD or APX activity. Tolerance of cold and oxidative stress was found to correlate with the antioxidant capacity of the plants, with linear correlations between leaf antioxidant capacity and the cold-induced decrease in photosynthetic parameters (Fig. 5). These results indicated that the generally high overall level of antioxidant activity in the selected DH lines and the enhancement of antioxidant enzyme activity during cold stress could lead to better cold tolerance in maize plants.

In conclusion, under chilling stress conditions, low photosynthetic electron transport activity, resulting in the poor utilization of absorbed light energy, low heat dissipation efficiency and limited antioxidant capacity were observed in the hybrid and control DH plants. These processes promoted the formation of ROS under cold stress conditions. In the oxidative stress-tolerant plants, however, the elevated antioxidant capacity was able to protect the photosynthetic apparatus from oxidative damage, resulting in better chilling tolerance. GR and GST were found to play an important role in the protection against chilling stress in maize.

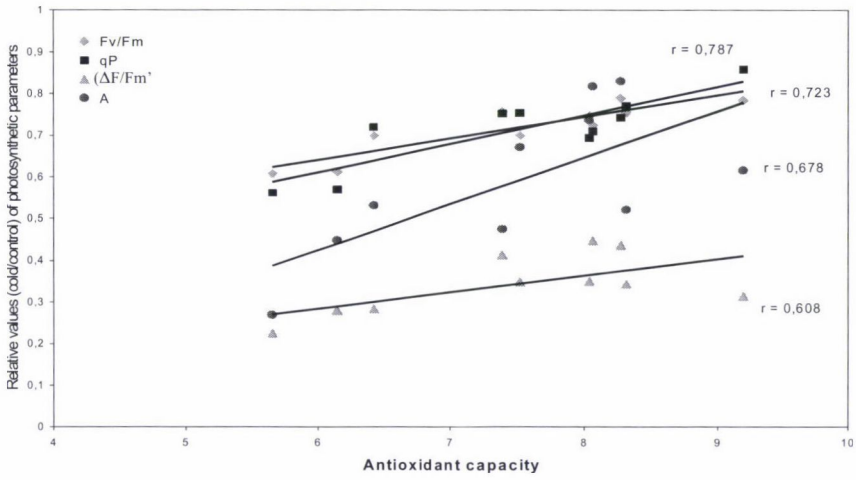

Fig. 5. Correlation between the photosynthetic activities measured by the optimal (Fv/Fm) and effective (ΔF/Fm') quantum yield of PS II, the photochemical quenching (qP) parameters the CO_2 assimilation rate (A) and the antioxidant capacities of the plants

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References

Allen, D. J., Ort, D. R. (2001): Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Sci.,* **6**, 36–42.

Ambrus, H., Darkó, É., Szabó, L., Bakos, F., Király, Z., Barnabás, B. (2006): *In vitro* selection in maize anther culture with oxidative-stress stimulators. *Protoplasma,* **228**, 87–94.

Apel, K., Hirt, H. (2004): Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Annu. Rev. Plant Physiol. Plant. Molec. Biol.,* **55**, 373–399.

Crafts-Brandner, S., Salvucci, M. (2000): Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. *Proc. Natl. Acad. Sci. USA,* **97**, 13430–13435.

Darkó, É., Ambrus, H., Fodor, J., Király, Z., Barnabás, B. (2009): Enhanced tolerance to oxidative stress with elevated antioxidant capacity in microspore derived DH maize plants. *Crop Sci.,* **49**, 628–636.

Darkó, É., Fodor, J., Dulai, S., Ambrus, H., Szenzenstein, A., Király, Z., Barnabás, B. (2011): Improved cold and drought tolerance of doubled haploid maize plants selected for resistance to prooxidant tert-butyl hydroperoxide. *J. Agron. Crop Sci.*, in press.

Fryer, M. J., Andrews, J. R., Oxborough, K., Blowers, D. A., Baker, N. R. (1998): Relationship between CO_2 assimilation, photosynthetic electron transport, and active O_2 metabolism in

leaves of maize in the field during periods of low temperature. *Plant Physiol.*, **116**, 571–580.

Janda, T., Kósa, E. I., Szalai, G., Páldi, E. (2005): Investigation of antioxidant activity in maize during low temperature stress. *Acta Biol. Szeged.*, **49**, 53–54.

Lee, E. A., Staebler, M., Tollenaar, M. (2002): Genetic variation and physiological discrimination for cold tolerance in maize (*Zea mays* L.). *Crop Sci.*, **42**, 1919–1929.

Lichtenthaler, H. K. (1987): Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Method Enzymol.*, **148**, 350–382.

Miller, G., Shulaev, V., Mittler, R. (2008): Reactive oxygen signaling and abiotic stress. *Physiol. Plant.*, **133**, 481–489.

van Kooten, O., Snel, J. (1990): The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynthesis Res.*, **25**, 147–150.

von Caemmerer, S., Farquhar, G. (1981): Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta,* **153**, 376–387.

Wilkinson, S., Clephan, A. L., Davies, W. J. (2001): Rapid low temperature-induced stomatal closure occurs in cold-tolerant *Commelina communis* leaves. *Plant Physiol.*, **126**, 1566–1578.

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RESPONSE OF MARTONVÁSÁR MAIZE INBRED LINES TO INCREASED UV-B RADIATION

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Solar UV-B radiation is generally regarded as an environmental stress factor, causing harm to living organisms by damaging DNA, proteins, lipids and membranes. Increased UV-B radiation may affect plant life directly or indirectly, having an influence on photosynthesis and plant biomass. In many plants, including maize (which is one of the most important crops in the world), exposure to increased UV-B radiation causes the induction of UV-B absorbing compounds (e.g. flavonoids), which act as UV-B screens and reduce the dangerous levels and effects of this radiation in plant tissues and cells.

This study aimed to reveal how Martonvásár maize inbred lines (bred under Central European environmental conditions) respond to increased UV-B radiation.

Key words: stress acclimation, UV-B radiation, antioxidants, flavonoids, anthocyanin, fluorescence imaging, UV-B phytotron chamber, *Zea mays* L.

Introduction

The level of UV-B radiation reaching the surface of the Earth has increased due to the thinning (or depletion) of the ozone layer in the stratosphere over recent decades (Teramura and Caldwell, 1981; Rozema et al., 1997; McKenzie et al., 1999). Without ozone life on Earth would be virtually impossible. Without its cooling effect the chemical bonds in DNA, which is the basis of all living organisms, would break up. A sufficiently thick ozone layer is the only thing that regulates the intense (though vital) energy of sunlight. Among the gases responsible for attenuating the ozone layer, chlorine, fluoride and bromide cause the most damage. On reaching the stratosphere a single molecule of these gases may destroy hundreds of thousands of ozone molecules.

When the ozone layer decreases by 1%, the UV-B radiation in sunlight increases by 2% (Mandronich, 1993). Based on the report of a 20-year survey by the U.S. Environmental Protection Agency (EPA) it can be established that the depletion of the ozone layer amounts to 2 or 3% per decade, though in some places it reached 15% (US EPA, 2010). This severely endangers the whole ecosystem, including both terrestrial and aquatic organisms. Dramatic changes have already been observed in the plankton.

The direct and indirect harmful effects of increased UV-B radiation on plants include the inhibition of photosynthesis, damage to the DNA, changes in morphology and phenology, a decrease in biomass accumulation, a reduction in the quantity, quality and viability of pollen, species-specific effects on growth and reproduction and an increase in the seed abortion rate (Teramura, 1983; Caldwell et al., 1989; Tevini and Teramura, 1989; Teramura and Sullivan, 1994; Walbot and Casati, 2006; Wang et al., 2008).

Most examinations on the negative effect of UV-B radiation have studied the vegetative rather than the generative parts of the plants (Demchik and Day, 1996). In the hundreds of plant species studied so far, radiation was found to cause a decrease in photosynthesis, while stimulating defence mechanisms, as manifested by visible changes (e.g. pigmentation) or measurable parameters (e.g. flavonoids as antioxidants). Nowadays the physiological reactions of plants are being increasingly analysed in order to learn how cultivated plants tolerate increases in harmful abiotic stress factors (such as high UV-B radiation) as a result of global climate change (Caldwell et al., 2007).

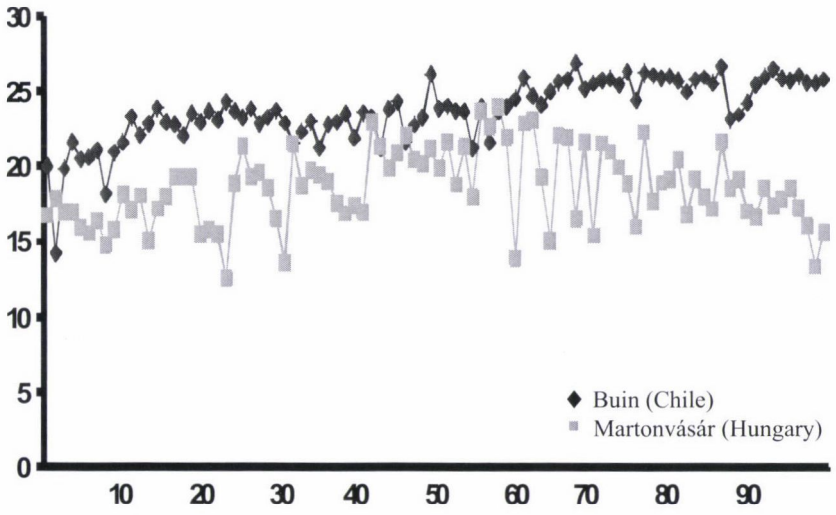
Besides high yield and quality, breeding germplasm resistant or tolerant to various biotic and abiotic stress factors is also important for almost all cultivated species. We may still be far from producing plants tolerant to UV-B radiation using molecular biotechnological methods, but experts all over the world are clear that efforts must be made to estimate the physiological and genetic reactions of the most important crops (e.g. wheat, soybean, maize) to increased UV-B radiation in field experiments, and to use this information in future plant breeding.

The population of the Earth will exceed 7 billion by 2012. The UN has recently published medium-term and long-term growth rates showing that the population will reach 9 billion by 2050 (source: United Nations, 2010). This means that 25% more food must be produced on the same area of land, which is an enormous challenge for agriculture. The production of the huge amounts of extra food required to satisfy increasing consumer demands also leads to the increased pollution of the environment. The governments, scientific institutions and international organizations responsible for the world population cannot ignore the unfavourable effects of climate change on agricultural production.

If the rate of air pollution does not decrease in the foreseeable future and if the countries of the world do not join forces to find a solution, then the further depletion of the ozone layer will have a catastrophic effect on the whole biosphere.

In the future this harmful effect of radiation cannot be ignored. Genetic resources, selection and screening methods must be found to develop parental

maize inbred lines capable of passing on tolerance to high UV-B radiation to their hybrids.



MED: minimum erythema dose;
1 MED=21 mJ/m^2

Fig. 1. Biologically effective UV irradiation on the basis of 5 years (2000–2004) in Buin (Chile) in November, December and January and in Martonvásár (Hungary) in May, June and July

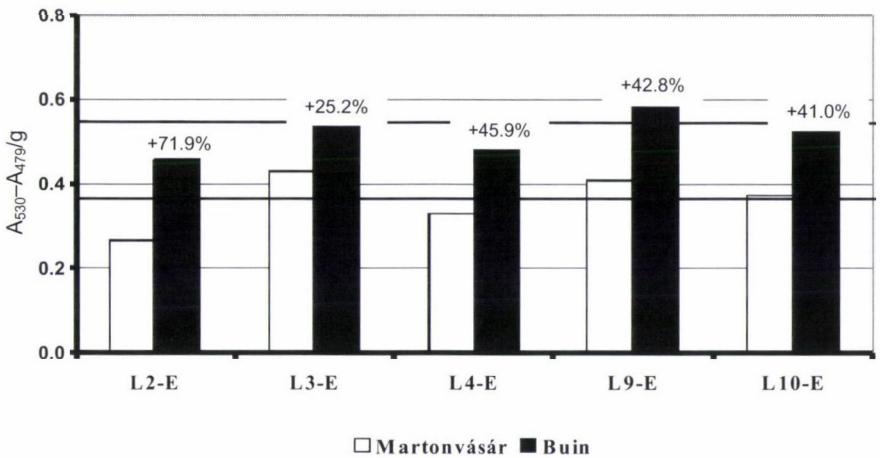


Fig. 2. Anthocyanin absorbance values of maize samples in Buin (Chile) and Martonvásár (Hungary) averaged over three years

At the Maize Breeding Department of the Agricultural Research Institute of the Hungarian Academy of Sciences, the selection and breeding of new inbred lines and hybrids involves the development of tolerance to various biotic and abiotic stress factors, including high UV-B radiation, to which inbred lines are very sensitive. This became clear when they were tested in a winter nursery in Chile, which has been used for the last 15 years.

In Chile the UV-B radiation is approximately 30–35% greater than in Hungary (Fig. 1) during the vegetation period of breeding materials, leading to a high proportion of pollen mortality, flowering asynchrony, barrenness and seed abortion.

Materials and methods

In the course of the experiment the same set of inbred lines, each having different maturity periods (5 early and 5 medium-late) and genetic backgrounds, were tested for five years in Chile and Hungary in four replications at each location. After the plants had flowered, the 3–4th leaf below the tassel was removed and stored at –80°C until required. The anthocyanin content was determined using a UV-VIS spectrophotometer (Shimadzu 160-A, Japan). The absorbance of the supernatant containing the anthocyanins, recorded at 530 nm, was corrected with the non-specific absorbance recorded at 479 nm. The differences between these absorbance values are illustrated in the figures. A compact flash-lamp fluorescence imaging system (FX-300UV) was used to obtain fluorescence images of the leaves. The fluorescence bands F440, F520, F690 and F740 were selected and four hundred images were accumulated for each sample. The images were processed using the Camille 1.05 image processing system. The UV-B radiation data were obtained from the Meteorological Services of Chile and Hungary. The AGROBASE'99 ANOVA program was used for statistical evaluation.

Results and discussion

The tests focused on anthocyanin, a flavonoid derivative involved in the absorption of damaging UV-B radiation. During this kind of environmental stress the level of reactive oxygen species (ROS) increases dramatically and this may cause significant damage to the cell structure.

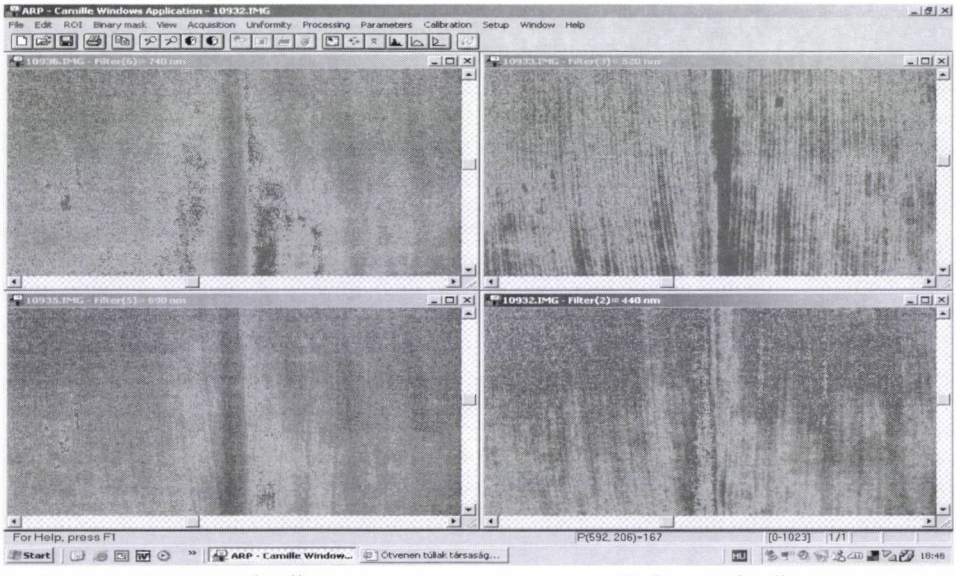
Averaged over years and varieties (maize inbred lines) the total anthocyanin content of the leaf samples was significantly higher in Chile than in Hungary (Fig. 2). This was presumably a response to the negative stress represented by higher UV-B radiation at the metabolic level. In the early-maturing flint lines the anthocyanin contents were more than 45% greater than those recorded in Hungary. This suggests that these genotypes, which were bred, developed and selected in northern regions (e.g. Canada), were not sufficiently adapted to the higher UV-B radiation level, resulting in a sharp rise in the quantity of anthocyanin (which absorbs the dangerous radiation) in these samples during exposure to increased UV-B radiation. In the late-maturing genotypes the initial content of the protective compound anthocyanin was higher at both locations, so in these types the high radiation level was not a problem and did not cause any substantial change in these experiments (Pintér et al., 2007).

Similar conclusions were drawn from the results obtained using a new method based on the fluorescence imaging technique (Fig. 3). The F440/F690 ratio (indicative of the stress level) was higher in late-maturing lines with high anthocyanin content, good tolerance and good adaptability to a higher level of UV-B radiation.

A special phytotron chamber was also used to determine the effect of higher UV-B radiation levels on photosynthesis. These measurements also confirmed the sensitivity of certain early maize genotypes, revealed by a reduction in net photosynthetic activity. Measurements on the activity of antioxidant enzymes confirmed that early inbred lines are more sensitive to UV-B radiation and were less able to eliminate reactive oxygen species and free radicals than later genotypes.

Measurements were made on the thickness, structure and waxiness of the epidermis, which also plays an important role in defence mechanisms. All these experiments and examinations provide direct information for maize breeders and help them produce new inbred lines that can be used as the parents of new hybrids with better tolerance to increased levels of UV-B radiation, one of the most dangerous consequences of global climate change (Wang et al., 2008).

Nowadays environmental factors are changing throughout the world. These results may contribute to the solution of the biggest challenge of the 21st century: to provide the continuously growing human population with food via sustainable agriculture in a permanently changing but unfortunately not computable ecosystem. It is clear from the results that breeders need to consider the effects of increased levels of UV-B radiation when breeding new varieties resistant to abiotic stress factors.



Late-maturing lines Early-maturing lines

Fig. 3. Fluorescence response of maize leaves

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References

Caldwell, M. M., Teramura, A. H., Tevini, M. (1989): The changing solar ultraviolet climate and the ecological consequences for higher plants. *Trends Ecol. Evol.*, **4**, 363–367.

Caldwell, M. M., Bornman, J. F., Ballaré, C. L., Flint, S. D., Kulandaivelu, G. (2007): Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. *Photochem. Photobiol. Sci.*, **6**, 252–266.

Demchik, S. M., Day, T. A. (1996): Effect of enhanced UV-B radiation on pollen quantity, quality and seed yield in *Brassica rapa* (Brassicaceae). *Am. J. Bot.*, **83**, 573–579.

Mandronich, S. (1993): UV radiation in the natural and perturbed atmosphere. pp. 17–69. In: Tevini, M. (ed.), *Effects of UV-B Radiation on Humans, Animals, Plants, Microorganisms and Materials*. Lewis Publishers, Boca Raton, FL.

McKenzie, R., Connor, B., Bodeker, G. (1999): Increased summertime UV radiation in New Zealand in response to ozone loss. *Science,* **285**, 1709–1711.

Pintér, J., Kósa, E., Hadi, G., Hegyi, Z., Spitkó,T., Tóth, Z., Szigeti, Z., Páldi, E., Marton, L. C. (2007): Effect of increased UV-B radiation on the anthocyanin content of maize (*Zea mays* L.) leaves. *Acta Agron. Hung.*, **55**, 7–17.

Rozema, J., Van de Staaij, J., Björn, L. O., Caldwell, M. M. (1997): UV-B as an environmental factor in plant life: Stress and regulation. *Trends Ecol. Evol.*, **12**, 22–28.

Teramura, A. H. (1983): Effects of ultraviolet-B radiation on the growth and yield of crop plants. *Physiol. Plant.*, **58**, 415–422.

Teramura, A. H., Caldwell, M. M. (1981): Effects of ultraviolet-B irradiances on soybean. IV. Leaf ontogeny as a factor in evaluating ultraviolet-B irradiance effects on net photosynthesis. *Am. J. Bot.*, **68**, 934–941.

Teramura, A. H., Sullivan, J. H. (1994): Effects of UV-B radiation on photosynthesis and growth of terrestrial plants. *Photosynth. Res.*, **39**, 463–473.

Tevini, M., Teramura, A. H. (1989): UV-B effects on terrestrial plants. *Photochem. Photobiol.*, **50**, 479–487.

United Nations (2010): *Population Facts* 2010/5. www.un.org/esa/population/publications/popfacts 2010-5.pdf (Last updated: 19.08.2010.)

US EPA (2010): Environmental Indicators: Ozone Depletion.

Walbot V., Casati, P. (2006): Impact of UV-B radiation on corn (*Zea mays* L.). *Energy Research at Stanford 2005–2006.*

Wang, Y., Qiu, N., Wang, X., Ma, Z., Du, G. (2008): Effects of enhanced UV-B radiation on fitness of an alpine species *Cerastium glomeratum* Thuill. *J. Plant Ecol.*, **3**, 197–202.

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BIOGAS PRODUCTION FROM SILAGE MAIZE HYBRIDS

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Experiments have been underway in Martonvásár for many years to develop leafy silage hybrids, which have a greater aboveground mass than conventional silage hybrids. The best hybrids for biogas production would be those that produce a large quantity of biomass and are rich in starch. The chief characteristic of leafy hybrids is that they have more leaves than normal hybrids. Due to this enhanced leaf area above the ear, the vegetative period of leafy genotypes is shorter, while the grain-filling period is longer, which has a positive effect on both yield and grain quality. The results of the present experiment show that during the anaerobic fermentation of the silage, leafy hybrids produced more biogas (640 l per 1000 g dry matter) than conventional hybrids (606 l per 1000 g dry matter). There were no significant differences between the methane contents of the leafy and non-leafy hybrids tested in the experiment. A strong positive correlation was found between biogas yield and the starch content of the silage, and a moderate positive correlation between biogas yield and the sugar content. The correlation between biogas yield and the lignin and protein contents was negative, in accordance with other literary data.

Key words: silage hybrids, leafy, non-leafy, biogas, quality, methane

Introduction

The existence of biogas has been known for many centuries. Shirley discovered marsh-gas in 1677 and Volta revealed it to be combustible in 1776 (Bai, 2007). The greatest advantage of biomass-based energy production is that it is a renewable energy source, which can be reproduced year by year. Biomass for energy purposes may originate directly from agricultural crop production, such as maize or potatoes. A common feature of energy plants is that they contain granular starch, which forms the raw material of fermentation. Maize, especially silage maize, could be one of the most important renewable energy sources, because it

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has a large dry matter yield, high protein and energy content and good digestibility, and the dry matter content at harvest is optimal for fermentation (Carter et al., 1991). The higher ratio of leaves in the total plant dry matter and the greater carbohydrate content in the leaves above the ear in leafy hybrids (Andrews et al., 2000) have a favourable influence on the quality and fermentability of the silage. The range of chemical components that need to be analysed is wider in the case of silage maize than for grain maize. The crude protein, crude ash, crude fat and crude fibre contents of the whole plant are also important for forage. Instead of starch content the water-soluble carbohydrate content (including mono- and oligosaccharides) is measured for silage. This influences fermentability to a great extent. Examples from other countries show that biogas plants based exclusively on silage maize are justified because the raw material production can easily be integrated into the existing agricultural system (Rácz et al., 2009). The results of experiments on silage maize hybrids show that leafy hybrids produce more biogas than conventional hybrids (Hegyi et al., 2009). Research has been performed to study how the maturity stage influences the amount of biogas extraction (Schittenhelm, 2008; Oslaj et al., 2010; Vindis et al., 2010). Late maturing "energy hybrids" benefit from the long maturity period and produce more biogas, provided that the dry matter content of the whole plant is high enough to produce good quality silage. Optimal plant density must be determined for the hybrids grown for biomass production in order to reach the optimal dry matter content of 27–32% (Jordanov, 1990). The objective of biogas production is to achieve a high concentration of methane in the fermentation end-product. Good quality biogas contains at least 60% methane (Herrmann and Taube, 2006). No significant correlation was found between the chemical composition and specific methane yield of the hybrids by Schittenhelm (2008), but other authors revealed that methane production depends on the composition of the biomass. Oslaj et al. (2010) found a significant correlation with crude protein content.

Materials and methods

An experiment on leafy and non-leafy silage hybrids (Table 1) was set up in a randomised complete block design with four replications on chernozem soil in Martonvásár, Hungary in 2009 and 2010. The experiment was sown with 31 seeds per row, which corresponds to 80,000 plants per hectare, with two rows per plot (plot size was 7.752 m^2). The morphological traits (plant height, main ear attachment height, length and width of the leaf next to the ear, leaf number above the main ear) were measured in the field after flowering on five plants from each treatment. The assimilating leaf area above the main ear was calculated using the equation reported by Montgomery (1906).

During August one row of each of the four leafy (Limasil, Dunasil, Mv Siloking, Mv Massil) and four non-leafy (Mv Maros, Mv NK 333, Mv TC 437, Maxima) varieties was cut, and chopped samples were prepared from the whole aboveground part of the plants. Part of each sample was used to analyse biogas yield in the BETA Research Institute in Sopronhorpács. Biogas formation consists fundamentally of two processes, fermentation and methane formation. During the phases of fermentation (hydrolysis, acidic phase) the large-molecule organic matter is decomposed with the help of enzymes and fermentation bacteria. The other part of each sample was measured by NIR spectroscopy and analysed using the "INGOT calibration of maize silage" software for chemi-

cal composition traits such as dry matter, ash, lignin, fat, starch and protein. This technique is also suitable for measuring *in vitro* digestibility. The NDF (neutral detergent fibre), WSC (water-soluable carbohydrate), NDICP (neutral detergent-insoluble crude protein), ADICP (acid detergent-insoluble crude protein) and lactic acid contents of the samples were also determined.

The data were statistically analysed using the ANOVA program Agrobase.

Table 1
Leafy and non-leafy silage maize hybrids tested in the experiment

Leafy silage hybrids	FAO number	Non-leafy silage hybrids	FAO number
Limasil	380	Mv Maros	330
Dunasil	390	Mv NK 333	390
Mv Siloking	580	Mv TC 437	440
Mv Massil	610	Maxima	580

Results and discussion

Plant height and leaf area

The mean height of the hybrids included in the experiment was 235.98 cm in 2009 and 286.89 cm in 2008. The plentiful rainfall in 2010 (681.1 mm in the vegetation period) allowed the plants to reach their genetically determined height, while the drought in 2009 (159.5 mm rainfall in the vegetation period) resulted in a mean height of 234.26 cm for non-leafy hybrids and 237.71 cm for leafy hybrids. In 2010, thanks to the large amount of rainfall during the growth period (May, June) an ideal height was achieved by both the leafy (291.53 cm) and non-leafy (282.25 cm) hybrids. These differences were statistically significant.

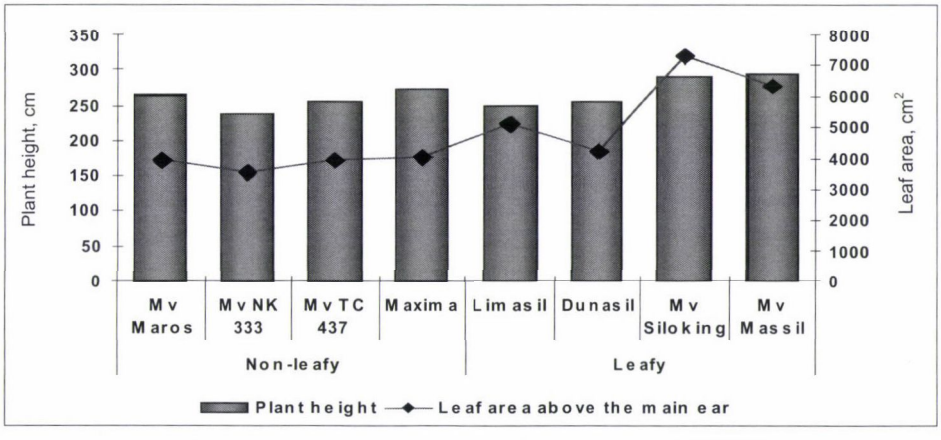

Fig. 1. Plant height (cm) and leaf area (cm^2) of leafy and non-leafy hybrids, averaged over 2009 and 2010

In both years the leaf area above the main ear was greater for leafy hybrids (non-leafy: 3854.13 cm^2; leafy: 5753.84 cm^2, averaged over the two years; Fig. 1).

This larger assimilation area was due to the greater number of leaves above the main ear (leafy: 8.71; non-leafy: 5.92) and to the greater width of the leaves (leafy: 11.16 cm; non-leafy: 10.73 cm). Averaged over two years, the young, actively photosynthesizing leaf area above the main ear ranged from 3540.13–4035.07 cm^2 for non-leafy hybrids and from 4244.20–7290.42 cm^2 for leafy hybrids.

Biogas and methane production

The biogas production of leafy and conventional hybrids was studied over two years. It was concluded that leafy hybrids produced more biogas during the anaerobic fermentation of silage maize (640 l per 1000 g dry matter) than conventional hybrids (606 l per 1000 g dry matter) (Table 2). The difference was statistically significant.

Table 2
Specific biogas yield from leafy and non-leafy hybrids (l per 1000 g dry matter) averaged over the years 2009 and 2010

Hybrid	Biogas, l/kg d.m.			Methane, %	
	2009	2010	Mean	2009	2010
Mv Maros	628	575	**602**	62.03	56.00
Mv NK 333	623	469	**546**	61.88	59.03
Mv TC 437	684	582	**633**	60.95	63.35
Maxima	719	566	**643**	59.23	58.23
Limasil	705	554	**630**	63.13	57.78
Dunasil	637	624	**631**	61.53	60.10
Mv Siloking	737	544	**641**	61.95	59.88
Mv Massil	733	585	**659**	60.43	61.38
Non-leafy mean	**664**	**548**	**606**	**61.02**	**59.15**
Leafy mean	**703**	**577**	**640**	**61.76**	**59.79**
$LSD_{5\%}$	**12.00**			**0.84**	

In both years the lowest biogas production was recorded for the same hybrid (Mv NK 333, 546 l per 1000 g dry matter), while a leafy hybrid (Mv Massil, 659 l per 1000 g dry matter) produced the greatest biogas yield (Fig. 2). The best quality biogas contains 60% methane. There were no significant differences between the methane contents of the leafy and non-leafy hybrids tested in the experiment. The process of outgassing took three weeks and the rate of outgassing was 87%, averaged over years and hybrids.

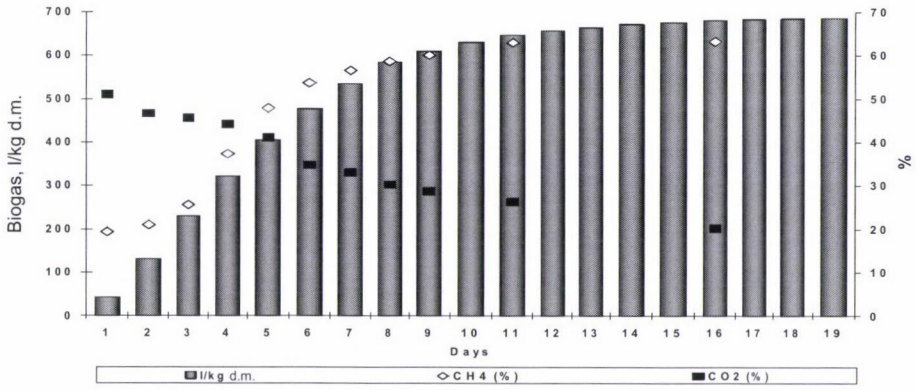

Fig. 2. Specific biogas yield from the maize hybrid Mv Massil, averaged over the years 2009 and 2010

Correlation between the chemical composition and the biogas yield

A strong positive correlation was found between the biogas yield and the starch content of the silage, and a moderate positive correlation between the biogas yield and the sugar content (Table 3). The correlation between the biogas yield and the lignin and protein contents was negative, in accordance with data in the literature.

Table 3
Chemical composition of the silage and the correlation with biogas yield, averaged over the years 2009 and 2010

Non-leafy	Biogas	Starch	Protein	Lignin	ADICP	NDF	NDICP	WSC
Mv Maros	601.50	36.56	8.80	4.44	4.13	53.60	2.42	5.41
Mv NK 333	546.00	32.93	9.70	4.24	4.22	53.40	2.40	5.48
Mv TC 437	633.00	34.33	8.50	4.15	4.17	54.48	2.28	5.36
Maxima	642.50	35.60	9.70	4.23	4.20	53.00	2.48	5.75
Mean	**605.75**	**34.86**	**9.18**	**4.27**	**4.18**	**53.62**	**2.40**	**5.50**
Correlation		0.59	–0.36	–0.23	–0.34	0.17	–0.04	0.31

Leafy	Biogas	Starch	Protein	Lignin	ADICP	NDF	NDICP	WSC
Limasil	629.50	36.18	8.90	4.27	4.17	54.75	2.35	5.04
Dunasil	630.50	36.50	9.49	4.02	4.07	54.80	2.38	5.57
Mv Siloking	640.50	35.40	8.82	4.21	4.20	53.17	2.51	5.95
Mv Massil	659.00	37.50	8.87	4.07	4.06	55.36	2.28	5.60
Mean	**639.88**	**36.40**	**9.02**	**4.14**	**4.13**	**54.52**	**2.38**	**5.54**
Correlation		0.61	–0.48	–0.32	–0.40	0.26	–0.38	0.41

Conclusions

In Western Europe areas removed from cultivation have been utilised for the production of renewable energy sources, which also has the effect of preventing rural migration. Hungary is poor in fossil fuels, but has good agricultural potential. Nevertheless, the number of families living chiefly from agriculture has gradually declined since EU accession. Nearly half a million people have had to give up growing food crops. The production, harvesting and processing of energy crops for the purpose of biogas production could provide jobs for these people, making it unnecessary for them to leave their homes and land. For a number of reasons, biogas production occupies a special place among renewable energy sources. This method of biomass utilisation is able to satisfy consumer demands in a complex manner, being suitable for heating, refrigeration and vehicle fuel purposes, while also resulting in valuable by-products (biofertiliser, carbon dioxide).

Official statistics show that the area sown to silage and fodder maize in Hungary has declined from 350,000 hectares in 1983 to 89,000 ha in 2008. Growing maize for biogas production could open up new prospects for this sector, especially if leafy silage maize hybrids were grown, which produce a larger quantity of biomass per unit area and have good fermentability, resulting in higher biogas yields. In the present experiments the biogas yield of leafy hybrids was significantly higher in both years than that of conventional hybrids.

Acknowledgements

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References

Andrews, C. J., Dwyer, L. M., Stewart, D. W., Dugas, J. A., Bonn, P. (2000): Distribution of carbohydrate during grainfill in leafy and normal maize hybrids. *Can. J. Plant Sci.,* **80**, 87–95.

Bai, A. (2007): *Biogáz.* (Biogas.) Száz magyar falu könyvesháza Kht., Budapest.

Carter, P. R., Coors, J. G., Undersander, D. J., Albrecht, K. A., Shaver, R. D. (1991): Corn hybrids for silage: an update. pp. 141–164. In: Wilkinson. D. (ed.), *Proc. of 46th Annual Corn and Sorghum Research Conference. Chicago, IL.* American Seed Trade Association, Washington, D.C.

Hegyi, Z., Tóthné Zsubori, Z., Rácz, F., Halmos, G. (2009): Comparative analysis of silage maize hybrids based on agronomic traits and chemical quality. *MAYDICA,* **54**, 133–137.

Herrmann, A., Taube, F. (2006): The use of maize for energy production in biogas plants – is research up to date with agricultural practice? *Berichte über Landwirtschaft,* **84**, 165–197.

Jordanov, J. (1990): Optimierung der Intensivierungfaktoren bei der Produktion von Mais. *Internationale Zeitschrift der Landwirtschaft,* **5**, 465–467.

Montgomery, E. G. (1906): What is an ear of corn? *Popular Sci. Mon.,* **68**, 55–62.

Oslaj, M., Mursec, B., Vindis, P. (2010): Biogas production from maize hybrids. *Biomass and Bioenergy,* **34**, 1538–1545.

Rácz, F., Hadi, G., Szőke, C., Tóth-Zsubori, Z., Hegyi, Z., Oross, D., Marton, L. C. (2009): Biogas production using silage maize hybrids bred in Martonvásár. In: *XXI. International Conference Eucarpia "Maize and Sorghum Breeding in the Genomics Era".* Bergamo, 21–24 June 2009, p. 132.

Schittenhelm, S. (2008): Chemical composition and methane yield of maize hybrids with contrasting maturity. *Eur. J. Agron.,* **29**, 72–79.

Vindis, P., Mursec, B., Janzekovic, M., Stajnko, D., Cus, F. (2010): Anaerobic digestion of maize hybrids for methane production. *Journal of Achievements in Materials and Manufacturing Engineering,* **40**, 87–94.

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ANALYSING SYMPTOMS OF *FUSARIUM* EAR ROT ON MAIZE (*ZEA MAYS* L.) USING AN *EX SITU* HYPERSPECTRAL EXAMINATION METHOD

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Kernel samples of two maize hybrids (46308 and 463017) with different levels of resistance to *Fusarium* ear rot were collected from artificially and naturally infected plants. The spectral characteristics of the samples were analysed with an ASD Fieldspec 3 MAX spectroradiometer in the wavelength range of 350 to 2500 nm using an *ex situ* method. The different extents of artificial and natural *Fusarium* infection on the maize kernels resulted in spectral differences detectable with a spectroradiometer. The data showed that for both genotypes the level of *Fusarium* infection generated by artificial inoculation was significantly higher than that caused by natural infection over a wavelength range of 2030 to 2080 nm. Principal Component Analysis (PCA) on the data set for this range revealed that the first component explained 77.0% of the variability for hybrid 46308 and 97.0% for hybrid 46317.

Key words: maize, ear rot, spectroradiometer, resistance breeding

Introduction

Fusarium ear rot is a fungal disease that is found worldwide wherever maize (*Zea mays* L.) is grown, so the control of this disease is the most important task for plant protection. Though other fungal species may also cause ear rot (Szécsi, 1994) in Hungary, maize ears are most frequently attacked by *Fusarium graminearum, F. verticillioides* and *F. culmorum* (Békési and Hinfner, 1970; Mesterházy, 1988). In addition to quantitative yield losses, all fungal species produce some form of mycotoxin as well. *F. culmorum* and *F. graminearum* chiefly produce deoxynivalenol (DON) and zearalenone (ZEA) (Thrane, 1989; Pettersson and Olvang, 1995), while *F. verticillioides* produces fumonisin and fusarin C (Chelkowski, 1998). Methods of diagnosing fungal diseases are divided

into two main groups, non-destructive and destructive. Destructive methods are based on chemical analysis. Methods based on gas and liquid chromatography and spectroradiometry are the most widespread, accurate techniques, but they are too time-consuming when analysing a large number of samples (Draganova et al., 2010). Non-destructive evaluation techniques provide information on product properties such as discontinuities and separations; structure; dimensions and metrology; physical and mechanical properties; composition and chemical analysis; stress and dynamic response; signature analysis and abnormal sources of heat (Giorleo and Meola, 2002). The potential of soft X-ray imaging to detect fungal infection in wheat was investigated by Narvankar et al. (2009). Healthy wheat kernels and kernels infected with the common storage fungi, namely *Aspergillus niger, A. glaucus* group, and *Penicillium* spp., were scanned using a soft X-ray imaging system. A two-class Mahalanobis discriminant classifier classified fungal-infected wheat kernels with 92.2–98.9% accuracy. According to Cleveland et al. (2008) many anatomical features of the kernels could be identified using neutron tomography. Kernels infected by *Aspergillus flavus* were found to have lower neutron attenuation in the scutellum and embryo regions. This phenomenon was possibly caused by lower hydrogen concentrations due to fungal degradation. Chelladurai et al. (2010) reported that the temperature profiles of fungus-infected samples of various wheat species were significantly different ($P < 0.05$) after heating for 180 s. The temperature profiles of the infected samples were also significantly different ($P < 0.05$) from those of healthy wheat samples. *Aspergillus flavus* and other pathogenic fungal species display typical infrared spectra which differ significantly from the spectra of substrate materials such as maize. On this basis, specific spectral features were identified by Gordon et al. (1997), which permit the detection of fungal infection on the surface of maize kernels by photoacoustic infrared spectroscopy. An approach for identifying *Fusarium*-infected single maize grains based on diffuse reflectance in the visible and near-infrared regions was detailed by Draganova et al. (2010). According to Shahin and Symons (2011), it can be concluded that hyperspectral imaging over 450–950 nm can be used to detect varying degrees of fusarium damage in CWRS wheat. Distinct differences between *Fusarium verticillioides*-infected and sound kernels were found by Williams et al. (2010) along the first principal component (PC) with two distinguishable clusters..

Visible and near-infrared reflectance and transmittance spectroscopy were used to detect fumonisin in single maize kernels infected with *Fusarium verticillioides*. Generally, models based on reflectance spectra have higher classification accuracy than models based on transmittance spectra (Dowell et al., 2002). A tabletop hyperspectral imaging system, VNIR-100E, was used by Yao et al. (2008). A total of five fungal species were selected for a two-part experiment: *Penicillium chrysogenum, Fusarium moniliforme* (syn.: *F. verticillioides*), *Aspergillus parasiticus, Trichoderma viride* and *Aspergillus flavus*. The results indicated that all five fungi were highly separable with a classification accuracy of 97.7%.

In the present work *ex situ* spectral analysis was carried out in the laboratory, where natural light was excluded, in order to study the symptoms of fungal disease caused by artificial *Fusarium* infection on maize genotypes. The fundamental spectral information obtained in this way will be supplemented with *in situ* field measurements in future studies.

Material and methods

In spring 2010 various genotypes were sown in replicated field plots in the pathological nursery of the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár. Artificial infection was carried out using a *Fusarium verticillioides* isolate. While the control plots were not infected artificially, the treated plants were infected using the toothpick method (Young, 1943) ten days after flowering. In autumn, prior to harvest, ear rot frequency and severity values were determined. Beyond the conventional examinations, kernel samples of two maize genotypes (46308 and 463017) with different levels of resistance to *Fusarium* ear rot were collected from artificially and naturally infected plants. The spectral characteristics of the samples were analysed with an ASD Fieldspec 3 MAX spectroradiometer in the wavelength range of 350 to 2500 nm using an *ex situ* method. The measurements were made in the Hungarian Institute of Agricultural Engineering, in a unique light-isolated cabinet (Fig. 1) which makes it possible to achieve outstanding precision, since environmental light is excluded and the interior of the measuring cabinet is designed to minimize undesirable reflection. The measuring process is presented in Figure 2. The special materials from which the sample alignment stage and the cabinet interior are made result in minimal reflectance over the whole electromagnetic spectrum detected by the spectroradiometer. Non-destructive contact measurements on the kernels were carried out with a "Plant Probe" sensor head (Fig. 3).

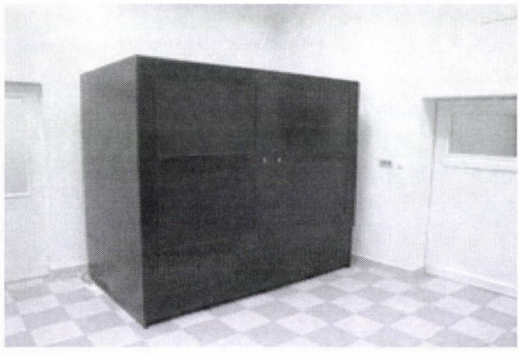

Fig. 1. Light-isolated cabinet

Fig. 3. Plant probe sensor head

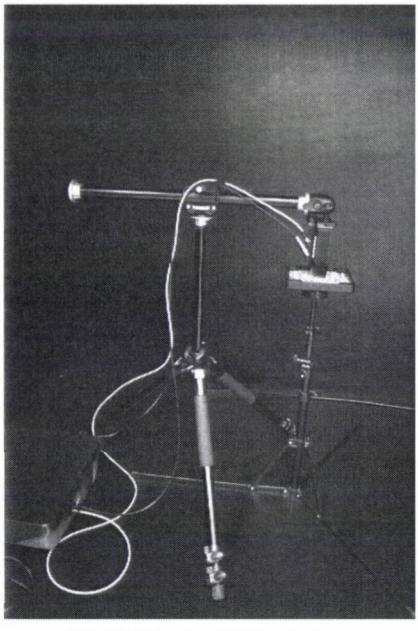

Fig. 2. The measuring process

The measurements were made in three replications for each kernel sample by recording ten spectra from each randomly selected sampling position. Each spectrum was the mean of fifty scans. Prior to recording each target spectrum a new white reference was taken. The integration time was set to 34 ms. The reflectance values recorded for each sample originated from the irradiation emitted by the light source in the "Plant Probe" sensor head and from that reflected from the surface of the maize kernels. The preliminary processing of the data was performed using ViewSpecPro software, while further processing steps were carried out with an ENVI image analyser and MS Excel software.

Results

In the course of the spectroradiometrical evaluation of the maize kernels the mean reflectance spectra of the treatments were computed for both hybrids (Figs. 4 and 5). Dashed lines represent artificial infection and solid lines naturally infec-

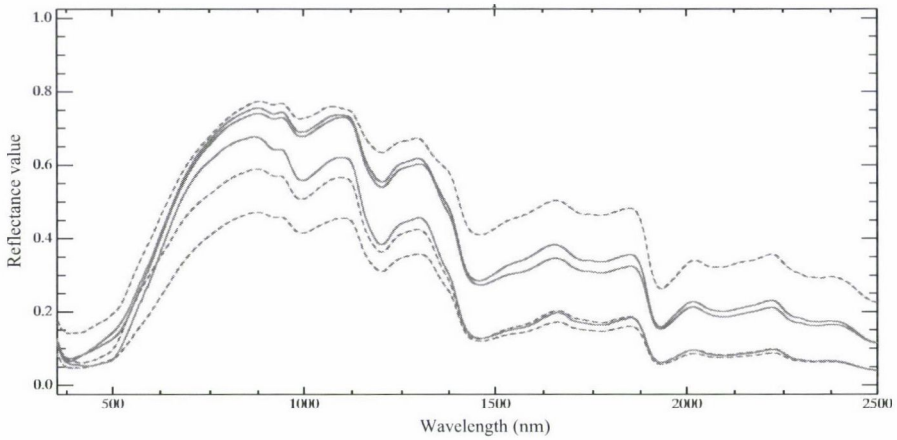

Fig. 4. Reflectance spectra of hybrid 46308

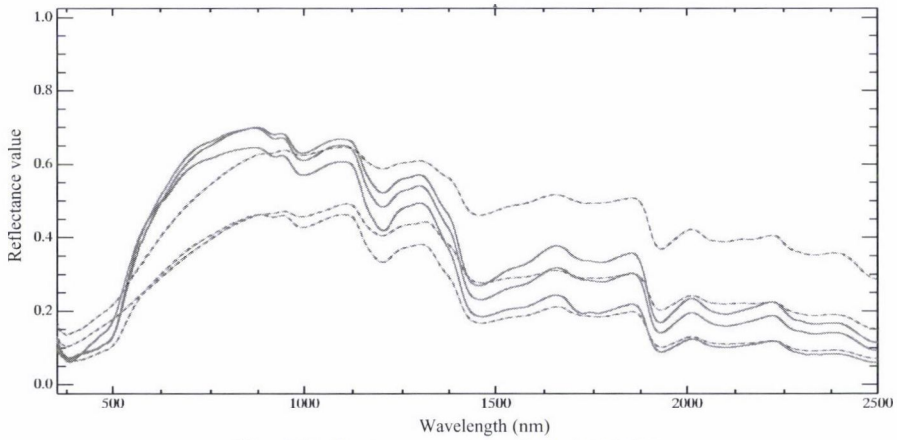

Fig. 5. Reflectance spectra of hybrid 46317

tion. According to these curves the spectral characteristics of the variants of the two hybrids diverged, but the deviation appeared to be independent of the level of infection.

In order to compare the individual absorption features from a common baseline the reflectance spectra were normalized and continuum removal was carried out by dividing the reflectance spectra using a convex hull fitted to the local minima (i.e. absorption maxima) of the reflectance curve. The results of continuum removal are presented in Figures 6 and 7.

After continuum removal, curve transformation was performed by forming the first derivative of the datasets. This revealed a characteristic interval between 2030 nm and 2080 nm, where the treated and untreated variants clearly diverged for both genotypes (Figs. 8 and 9).

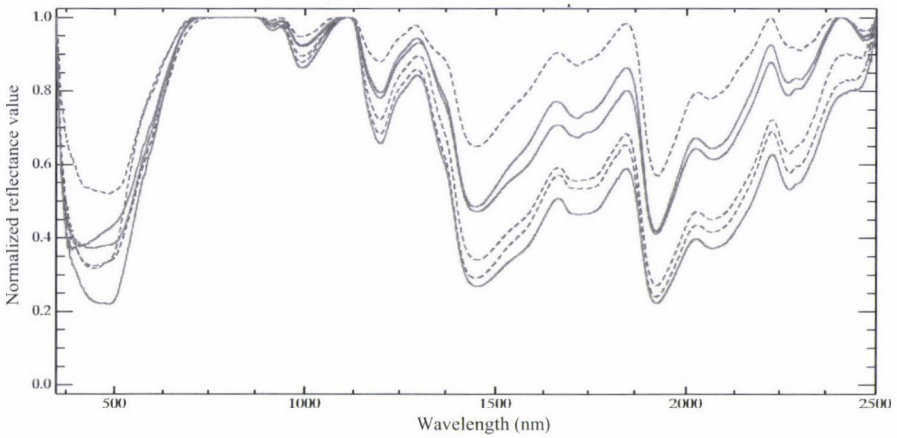

Fig. 6. Curves obtained after continuum removal for genotype 46308

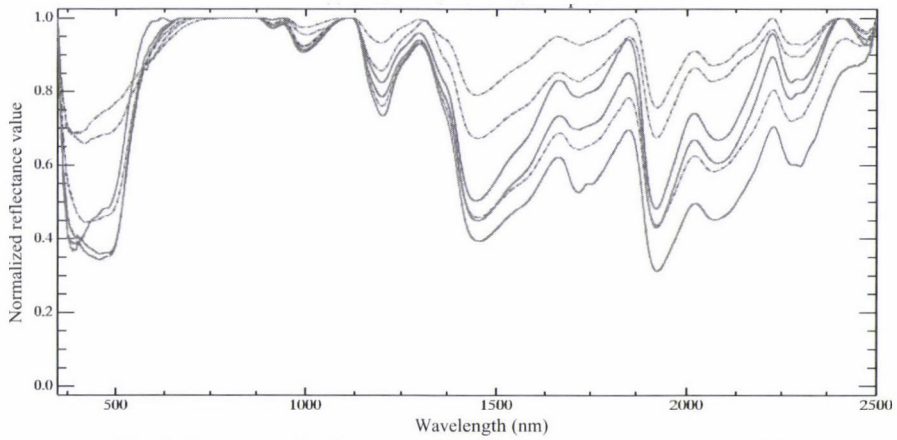

Fig. 7. Curves obtained after continuum removal for genotype 46317

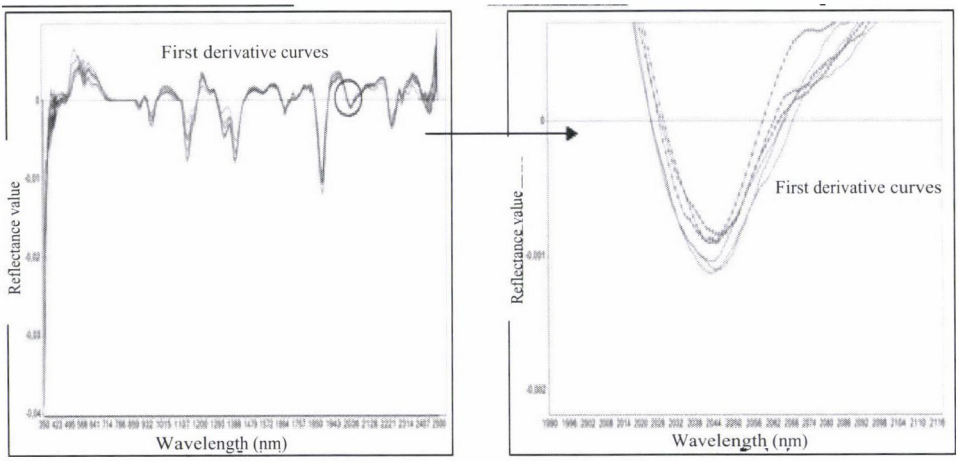

Fig. 8. First derivative curves for genotype 46308, with the informative wavelength range highlighted

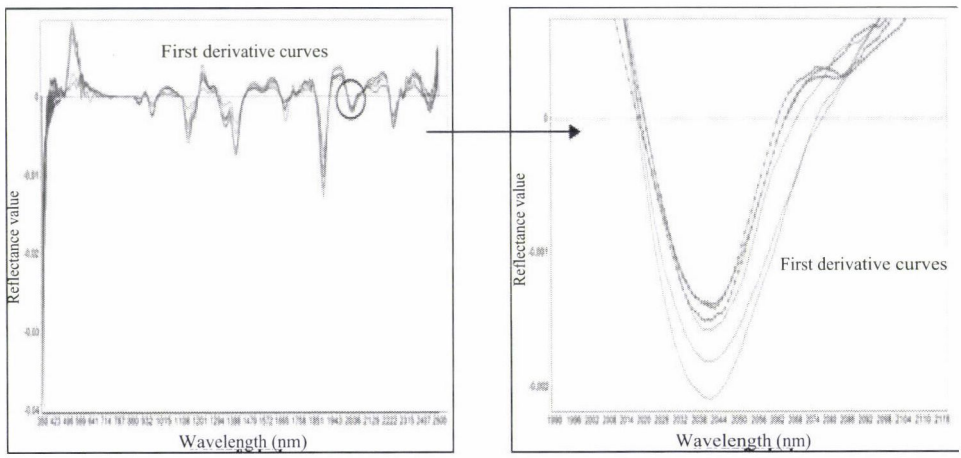

Fig. 9. First derivative curves for genotype 46317, with the informative wavelength range highlighted

When Principal Component Analysis was executed on this interval (Figs. 10 and 11) the plots of the Principal Component score values showed a pronounced separation between the samples of artificially infected and naturally infected hybrids, indicating that they also differed spectrally. While the first component explained 77% of the variability for hybrid 46308, this figure was 97% for genotype 46317.

The stronger correlation indicated by PCA in the case of hybrid 46317 suggests a greater difference between the artificially infected and naturally infected variants than in hybrid 46308. In order to quantitatively compare the *Fusarium* se-

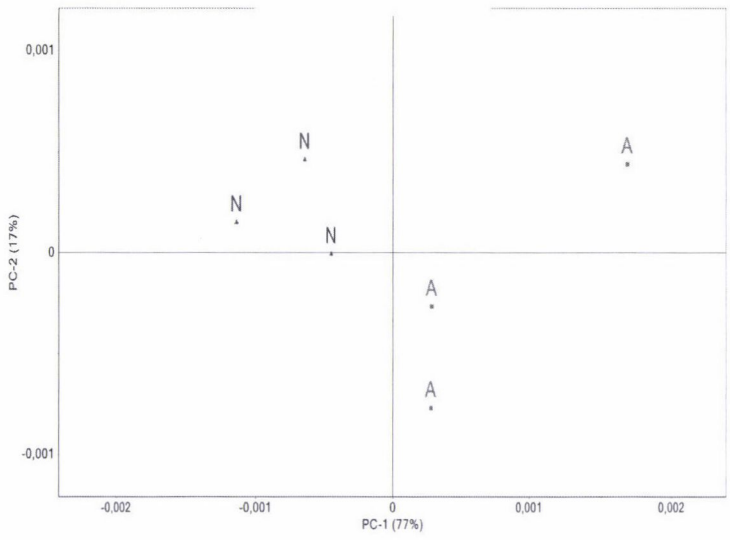

Fig. 10. Principal Component Analysis for genotype 46308
(A = artificial, N = natural infection)

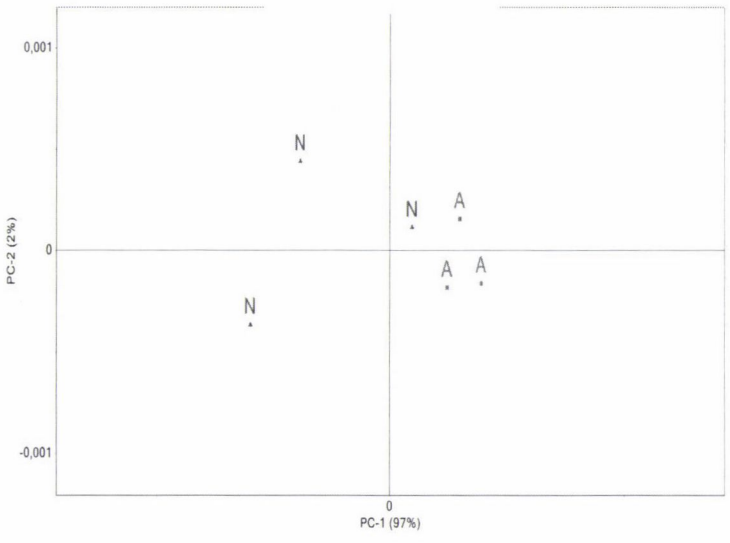

Fig. 11. Principal Component Analysis for genotype 46317
(A = artificial, N = natural infection)

verity of the samples, a conventional evaluation of the kernels was carried out for both genotypes. The data are summarized in Table 1.

Table 1
Conventional evaluation of the kernels according to *Fusarium* severity

Hybrid ID	Treatment	Treatment code	*Fusarium* severity (%)
46308	Artificially infected	A	8.889
46308	Naturally infested	N	1.778
46308	Artificially infected	A	10.000
46308	Naturally infested	N	9.444
46317	Artificially infected	A	17.143
46317	Naturally infested	N	1.778
46317	Artificially infected	A	36.593
46317	Naturally infested	N	7.000

According to the severity data, genotype 46308 was only affected moderately by artificial infection, while artificial infection resulted in intense *Fusarium* symptoms in hybrid 46317.

Discussion

The different extents of artificial and natural *Fusarium* infection on the maize kernels resulted in spectral differences which were detectable with a spectroradiometer. Based on the data, the higher level of *Fusarium* infection generated in both genotypes by artificial inoculation could be statistically distinguished from that caused by natural infection in the wavelength range of 2030 to 2080 nm. In this range both maize hybrids showed the same spectral features for the two infection methods applied. In order to validate the method and to improve the evaluation process, further maize hybrids and more *Fusarium* species will be studied in experiments on artificial inoculation. The physiological changes caused by kernel infection in individual kernels of different genotypes (tolerant, average and susceptible) will be examined under laboratory conditions. Kernels placed on PDA medium in Petri dishes will be inoculated with *Fusarium* species *(F. graminearum, F. verticillioides)* after external and internal kernel disinfection. Disinfected kernels will be evaluated as a control. The spectral features will be studied using the method outlined above. If the interrelation between different extents of *Fusarium* infection is clarified, spectral analysis could be of great assistance in plant protection and in resistance breeding.

Acknowledgements

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References

Békési, P., Hinfner, K. (1970): Adatok a kukorica fuzáriumos eredetű megbetegedéseinek ismeretéhez. (Contribution to the knowledge of maize diseases of *Fusarium* origin.) *Növényvédelem,* **6**,13–18.

Chelkowski, J. (1998): Distribution of *Fusarium* species and their mycotoxins in cereal grains. pp. 45–64. In: Sinha, K. K., Bhatnagar, D. (eds.), *Mycotoxins in Agriculture and Food Safety*. Marcel Dekker Inc., New York.

Chelladurai, V., Jayas, D. S., White, N. D. G. (2010): Thermal imaging for detecting fungal infection in stored wheat. *J. Stored Prod. Res.,* **46**, 174–179.

Cleveland, T. E. IV, Hussey, D. S., Chen, Z.-Y., Jacobson, D. L., Brown, R. L., Carter-Wientjes, C., Cleveland, T. E., Arif, M. (2008): The use of neutron tomography for structural analysis of corn kernels. *J. Cereal Sci.,* **48**, 517–525.

Dowell, F. E., Pearson, T. E., Maghirang, E. B., Xie, F., Wicklow, D. T. (2002): Reflectance and transmittance spectroscopy applied to detecting fumonisin in single corn kernels infected with *Fusarium verticillioides*. *Cereal Chem.,* **79**, 222–226.

Draganova, T., Daskalov, P., Tsonev, R. (2010): An approach for identifying of *Fusarium* infected maize grains by spectral analysis in the visible and near infrared region, SIMCA models, parametric and neural classifiers. *Int. J. Bioautomat.,* **14**, 119–128.

Giorleo, G., Meola, C. (2002): Comparison between pulsed and modulated thermography in glass-epoxy laminates. *NTD & E International,* **35**, 287–292.

Gordon, S. H., Schudy, R. B., Wheeler, B. C., Wicklow, D. T., Greene, R. V. (1997): Identification of Fourier transform infrared photoacoustic spectral features for detection of *Aspergillus flavus* infection in corn. *Int. J. Food Microbiol.,* **35**, 179–186.

Mesterházy, Á. (1988): Expression of resistance to *Fusarium graminearum* and *F. culmorum* under various experimental conditions. *J. Phytopathol.,* **133**, 304–310.

Narvankar, D. S., Singh, C. B., Jayas, D. S., White, N. D. G. (2009): Assessment of soft X-ray imaging for detection of fungal infection in wheat. *Biosystems Engineering,* **103**, 49–56.

Pettersson, H., Olvang, H. (1995): Trichothecene production by *Fusarium poae* and its ecology. In: *Proceedings of the International Seminar on Fusarium – Mycotoxins, Taxonomy and Pathogenicity.* Martina Franca, Italy, pp. 178–179.

Shahin, M. A., Symons, S. J. (2011): Detection of *Fusarium* damaged kernels in Canada Western Red Spring Wheat using visible/near-infrared hyperspectral imaging and principal component analysis. *Computers and Electronics in Agriculture,* **75**, 107–112.

Szécsi, Á. (1994): A *Liseola* szekcióba tartozó fuzáriumok előfordulása hazai kukoricakultúrákban 1991 és 1992. évben. (Occurrence of *Fusarium* species in section *Liseola* isolated from Hungarian corn samples in 1991 and 1992.) *Növényvédelem,* **30**, 313–318.

Thrane, U. (1989): *Fusarium* species and their specific profiles of secondary metabolites. pp. 199–225. In: Chelkowski J. (ed.), *Fusarium Mycotoxins, Taxonomy and Pathogenicity. Topics in Secondary Metabolism – Vol. 2*. Elsevier, Amsterdam.

Williams, P., Manley, M., Fox, G., Geladi, P. (2010): Indirect detection of *Fusarium verticillioides* in maize (*Zea mays* L.) kernels by NIR hyperspectral imaging. *J. Near Infrared Spec.,* **18**, 49–58.

Yao, H., Hruska, Z., Kincaid, R., Brown, R. L., Cleveland, T. E. (2008): Differentiation of toxigenic fungi using hyperspectral imagery. *Sens. & Instrumen. Food Qual.*, **2**, 215–224.

Young, H. C. (1943): The toothpick method of inoculating corn for ear and stalk rots. (Abstr.) *Phytopath.*, **33**, 16.

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EFFECTIVENESS OF MAJOR RESISTANCE GENES AND IDENTIFICATION OF NEW SOURCES FOR DISEASE RESISTANCE IN WHEAT

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Among the factors which determine yield reliability an important role is played by disease resistance. One of the breeding aims in the Martonvásár institute is to develop wheat varieties with resistance to major diseases. The winter wheat varieties bred in Martonvásár are examined in artificially inoculated nurseries and greenhouses for resistance to economically important pathogens. The effectiveness of designated genes for resistance to powdery mildew and leaf rust has been monitored over a period of several decades. None of the designated major resistance genes examined in greenhouse tests is able to provide complete resistance to powdery mildew; however, a number of leaf rust resistance genes provide full protection against pathogen attack (Lr9, Lr19, Lr24, Lr25, Lr28 and Lr35). In the course of marker-assisted selection, efficient resistance genes (Lr9, Lr24, Lr25 and Lr29) have been incorporated into Martonvásár wheat varieties. The presence of Lr1, Lr10, Lr26, Lr34 and Lr37 in the Martonvásár gene pool was identified using molecular markers. New sources carrying alien genetic material have been tested for powdery mildew and leaf rust resistance. Valuable *Fusarium* head blight resistance sources have been identified in populations of old Hungarian wheat varieties. Species causing leaf spots (*Pyrenophora tritici-repentis, Septoria tritici* and *Stagonospora nodorum*) have gradually become more frequent over the last two decades. Tests on the resistance of the host plant were begun in Martonvásár four years ago and regular greenhouse tests on seedlings have also been initiated.

Key words: wheat, resistance, fungal diseases, marker-assisted selection, breeding

Introduction

On a world scale wheat is attacked by 200–250 pathogens and pests. In Hungary there are 5–10 diseases which occur frequently and may cause significant economic losses in commercial wheat production. The most environmentally

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sound, low cost method of controlling wheat diseases is to breed and grow resistant wheat varieties.

Wheat powdery mildew [*Blumeria graminis* (D.C.) Speer f. sp. *tritici* Ém. Marchal] is widespread on wheat throughout the world. Although quantitative resistance to powdery mildew is widely used in resistance breeding, major resistance genes (Pm genes) are also present in the wheat gene pool. Fifty-nine genes/alleles conferring resistance to the pathogen have been identified so far (McIntosh et al., 2010). However, the vast majority of these genes have been overcome by the pathogen (Szunics et al., 2001).

Improving resistance to rust fungi (leaf, stem and stripe rust) is one of the major tasks facing wheat breeders all over the world. In Hungary the greatest damage is currently caused by leaf rust (*Puccinia triticina* Erikss.), which can be expected to infect wheat fields every year. The various strategies that can be applied in breeding for leaf rust resistance can be divided into two groups. The first is based on the use of designated leaf rust resistance genes (Lr genes), and involves the incorporation of effective, previously unexploited or underexploited Lr genes into varieties with a favourable agronomic background, and the combination of such genes at the plant or population level. The second group of strategies involves methods utilising horizontal resistance (Winzeler et al. 2000).

Efficient protection against *Fusarium* species could be achieved by growing wheat varieties tolerant to Fusarium head blight (FHB). Only a limited number of FHB resistance sources are currently available to breeders. At present spring genotypes of Far Eastern origin, especially Sumai 3 and its derivatives (e.g. CM82036), are considered to have the best resistance (Bai and Shaner, 1994), but the agronomic traits of these genotypes differ greatly from those of the winter wheat varieties cultivated in Hungary.

As other fungal species attacking the leaf area (powdery mildew, rust fungi) have been more effectively tackled, species causing leaf spots (*Pyrenophora tritici-repentis, Septoria tritici* and *Stagonospora nodorum*) have gradually become more frequent over the last two decades. Tests on the resistance of the host plant were begun in Hungary nearly ten years ago and in addition to field experiments, regular greenhouse tests on seedlings have also been initiated (Csősz et al., 2003; Cséplő et al., 2004). The results achieved up to now have indicated consistent differences in the resistance of individual wheat varieties.

The use of molecular markers in wheat breeding programmes is on the increase (Collard and Mackill, 2008). In the course of marker-assisted breeding, effective resistance genes can be incorporated and pyramided into wheat varieties with the help of PCR-based DNA markers (STS, SCAR, CAPS and SSR) and resistance genes can be easily identified.

Materials and methods

A set of differential cultivars proposed by COST Action 817 and two additional genotypes were used to test the effectiveness of 18 Pm resistance genes and gene combinations (Pm1, Pm2, Pm3a, Pm3b, Pm3c, Pm3d, Pm4a, Pm4b, Pm5, Pm6, Pm7, Pm8, Pm17, Pm1+2+9, Pm2+4b+8, Pm2+6, Pm3f and the susceptible check) in the seedling stage in the greenhouse between 2006 and 2010. The genotypes tested were inoculated under an isolator box on the 8th day after sowing (GS11) using single-pustule isolates. The isolates used in the greenhouse test were collected in Martonvásár from a number of varieties currently or previously grown on large areas in Hungary and from genotypes carrying designated Pm genes or gene combinations. Prior to infection the powdery mildew population was multiplied on the variety Carstens V (Pm0). The level of seedling infection was scored on a 0–4 scale (0–2 = resistant, 3–4 = susceptible) after ten days. A new genetic source, an introgression breeding line (34-CSXV32-V616-1-R) from Viterbo (Italy) originating from a wheat × *Dasypyrum villosum* combination, was tested for powdery mildew resistance under field and greenhouse conditions.

The effectiveness of major leaf rust resistance genes was evaluated in an artificially inoculated nursery. The leaf rust resistance of 36 Thatcher-based near-isogenic lines was evaluated. Rows of a spreader variety, planted around the tested genotypes, were inoculated in development stage 37–39 on the Zadoks scale (Zadoks et al., 1974) using the uredospore mixture. The spore suspension was injected into the spreader plants using a hypodermic syringe. The pathogen then spread naturally from these primary sources of infection. The extent of infection at development stage 77–83 was evaluated in terms of severity (according to the modified Cobb scale; Stubbs et al., 1986) and host response (resistant, moderately resistant, intermediate, moderately susceptible and susceptible). The average coefficient of infection (ACI) was calculated from these two data by multiplying the severity by an assigned constant value for the host response, for use in the statistical evaluation (Stubbs et al., 1986).

A backcross programme was carried out, aimed at creating new sources for leaf rust resistance by transferring effective Lr genes (Lr9, Lr24, Lr25 and Lr29). Martonvásár winter wheat varieties (Mv Emma, Mv Madrigál, Mv Pálma and Mv Magvas) were crossed with resistance sources. The F_1 plants were backcrossed to the recurrent parents. BC_1 plants were selected by means of marker-assisted selection from different backcross generations and these were again backcrossed to the recurrent parent.

The presence of five leaf rust resistance genes (Lr1, Lr10, Lr26, Lr34 and Lr37) was analysed in the Martonvásár wheat pool. Molecular markers WR003 for Lr1 (Qiu et al., 2007), ThLr10 for Lr10 (Feuillet et al., 2003), IAG95 for Lr26 (Mohler et al., 2001), csLV34 for Lr34 (Lagudah et al., 2006) and SC-Y15 for Lr37 (Robert et al., 1999) were applied using the published PCR protocols.

Field experiments artificially inoculated with *Fusarium culmorum* were set up in five years on 98 populations and lines of old Hungarian varieties, together with two control varieties (Sumai 3, resistant, and GK Zugoly, susceptible). Conidial suspensions were used to spray-inoculate the plants at 50% flowering, and the inoculations were repeated two days later. The spore concentration applied was 5×10^4 macroconidia ml^{-1}. Mist irrigation was applied. As a measure of FHB severity the ratio of *Fusarium*-infected spikelets was determined by visually scoring the inoculated plot on the 26th day after the first inoculation.

In studies on *Pyrenophora tritici-repentis* greenhouse experiments were set up on Martonvásár wheat varieties, breeding lines and genotypes with known genetic background. The genotypes were inoculated with an isolate of tan spot (Pti 2) developed on V8PDA medium. The inoculum was sprayed onto the leaf surface when the plants were in the 1-leaf stage. The genotypes were evaluated from the 5th day after inoculation, scoring the lesion types on a 1–5 scale (1 = resistant, 5 = susceptible) (Lamari and Bernier, 1989). The area under the disease progress curve (AUDPC) was calculated from the lesion type values recorded at various dates (Shaner and Finney, 1977).

Results and discussion

The virulence of the wheat powdery mildew population to 18 Pm genes and gene combinations was analysed in five years (2006–2010). In the seedling test, none of the genes examined was able to ensure complete resistance to powdery mildew (Table 1). This was true even of the Pm3b gene, for which only 24.8% of the isolates exhibited virulence. Less than 50% of the isolates were able to infect

Table 1
Virulence of the wheat powdery mildew population on differentials
Martonvásár, 2006–2010

Differential	Pm gene	Virulence, %				
		2006	2007	2008	2009	2010
Carsten V.	0	100.0	100.0	100.0	100.0	100.0
Axminster/8*CC	1	55.6	59.1	53.2	49.7	57.4
Ulka/8*CC	2	91.8	91.8	90.3	94.7	94.1
Asosan/8*CC	3a	70.4	79.3	79.0	83.4	60.4
Chul/8*CC	3b	21.9	26.0	28.0	26.2	21.9
Sonora/8*CC	3c	94.4	97.6	100.0	97.3	97.6
Ralle	3d	43.9	36.1	38.2	23.0	23.7
Mich. Amber/CC*8	3f	42.4	35.1	36.0	45.5	42.4
Khapli/8*CC	4a	100.0	99.5	100.0	100.0	100.0
Ronos	4b	76.0	85.9	87.6	85.6	93.5
Rektor	5	100.0	99.0	100.0	100.0	89.4
NK-747	6	90.8	97.6	98.4	100.0	99.4
Transfed	7	74.5	95.6	97.8	98.4	99.4
Disponent	8	100.0	99.5	100.0	100.0	100.0
Amigo	17	62.2	88.5	93.0	93.1	95.3
Maris Huntsman	2+6	77.0	86.0	88.7	94.7	92.3
Apollo	2+4b+8	66.3	81.7	79.6	79.7	66.3
Normandie	1+2+9	65.8	89.9	52.2	40.1	65.8

$LSD_{5\%} = 9.4$

differentials carrying the Pm3d (33.0%) and Pm3f (40.2%) genes. In the case of varieties bearing genes Pm4a, Pm8, Pm5, Pm3c, Pm2, Pm6, Pm7 and Pm2, the virulence of the powdery mildew population did not differ significantly from the value observed for the susceptible control (100%). According to earlier observations (Vida et al., 2007), in field experiments on adult plants the resistance of the variety Ralle, which carries the Pm3d gene, is outstandingly good at present, and favourable results have also been obtained with wheat genotypes carrying the Pm21 resistance gene. The donor of the Pm21 gene is *Dasypyrum villosum,* a wild relative of wheat. In the framework of a bilateral research project the powdery mildew resistance of an introgression breeding line carrying alien genetic material from *D. villosum* was tested for two years under field conditions in the adult plant stage and for one year in two independent experiments in the greenhouse, in the

seedling stage. All the experiments proved the outstanding resistance of the line. Earlier studies confirmed the presence of a dominant gene in the genetic background of the introgression line (Bizzarri et al., 2007), but it has not been proved whether it is the Pm21 gene itself, or an allelic variant of it. Further studies are required to clarify whether this gene is really a putative example of non-host resistance, as proposed by Bizzarri (2009), or just a highly effective, but vulnerable major gene. However, neither in Hungary nor elsewhere is the one-sided exploitation of major resistance genes the aim of resistance breeders. If wheat varieties carrying one of these genes were grown on large areas, it is highly likely that pathotypes virulent to the genes would multiply very rapidly, making the resistance of these varieties worthless.

The field resistance of wheat genotypes carrying designated Lr genes has been tested for several decades in order to determine the efficiency of major leaf rust resistance genes. Each year Thatcher-based near-isogenic lines, each carrying a different resistance gene or allele, are sown in the experiments. The results indicate that eight of the near-isogenic lines (NIL) carrying a single Lr gene or allele are still not infected with the pathogen, or only to a negligible extent (Fig. 1). Wheat lines carrying the genes Lr9, Lr19, Lr24, Lr25, Lr28, Lr29 and Lr35 had excellent leaf rust resistance in Martonvásár, averaged over the last 8 years. Moderately susceptible (30–40MS) and susceptible (80S) reactions were detected in 2009 and 2010, respectively, on wheat genotypes carrying the Lr37 gene in their genetic background, although there was no sign of infection on them earlier. The line exhibiting the greatest degree of infection was the NIL carrying gene Lr14a.

A marker-assisted backcross programme was set up to incorporate leaf rust resistance genes into four Martonvásár wheat varieties. Up till now lines in the BC_5–BC_6 generation have been developed for various crosses. Since the use of Lr

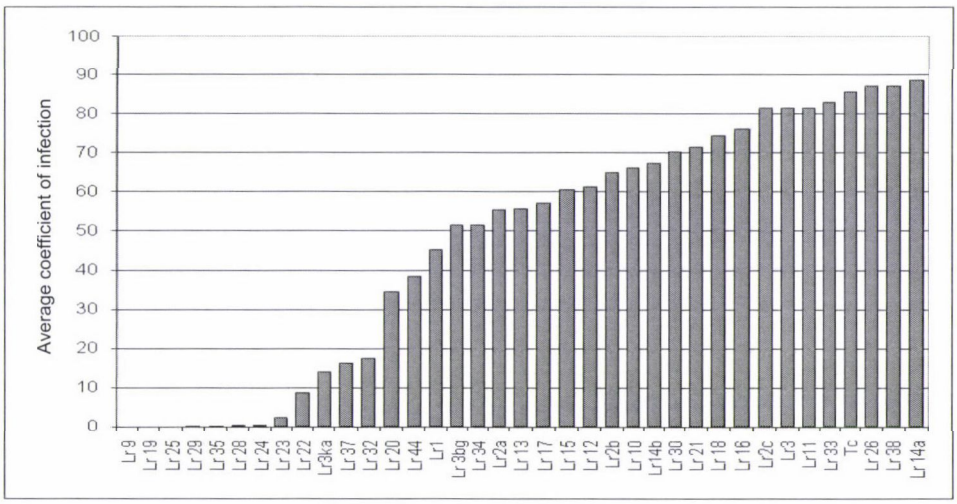

Fig. 1. Leaf rust resistance of Thatcher-based near-isogenic lines Martonvásár, mean of 2004–2010

genes singly increases the danger of genetic vulnerability, combinations of lines carrying different genes were developed in order to pyramid the genes. The aim was to create genotypes carrying several resistance genes simultaneously, in the hope that these would have more durable resistance to leaf rust than those carrying a single gene. To date, a number of gene combinations have been developed for the four Martonvásár varieties. A doubled haploid programme has been set up based on anther culture in order to stabilise the gene combinations.

Designated leaf rust resistance genes were identified in Martonvásár wheat varieties using molecular markers (Table 2). The Lr1 and Lr10 genes were assumed to be present in the Martonvásár gene pool, but this was the first time it could be proved using molecular markers (11 and 15 out of 74 Mv varieties, respectively). During investigations aimed at detecting designated resistance genes, a reduction in the proportion of varieties carrying the Lr26 resistance gene was noted among wheat varieties registered in recent years. The 1BL.1RS translocation was present in 77.1% of the 35 varieties released before 2000, while this figure had dropped to 37.5% in the 24 most recently registered genotypes. Pedigree analysis on the Martonvásár wheat varieties demonstrated that the variety Bezostaya 1, or its ancestor Bezostaya 4 – both of which carry the Lr34 gene, was present in the pedigree of almost all the varieties. As expected, the Lr34 gene was found in many Martonvásár varieties (12 varieties). The Lr37 resistance gene can be detected at high frequency in the genome of Western European wheat varieties, but only one Mv variety (Mv Vekni) carries this gene.

Table 2
Number and ratio of Martonvásár winter wheat varieties with designated leaf rust resistance genes

	Lr1	Lr10	Lr26	Lr34	Lr37
Number of Martonvásár varieties	11	15	59	12	1
Ratio, %	14.9	20.3	79.8	16.2	1.4

The results of analysis of variance demonstrated that the mean field FHB infection of old Hungarian wheat varieties and lines was significantly influenced by the year. The most severe infection was recorded in 2004 (45.3%), followed by 2007 (37.3%), 2003 (36.4%), 2006 (28.6%) and 2005 (21.6%, $LSD_{5\%} = 4.7\%$). Significant differences were also observed between the lines. The FHB infection of the wheat lines and varieties fluctuated over a wide range (Sumai 3 = 2.8%; GK Zugoly = 86.0%), averaged over the five years. The FHB infection of old Hungarian wheat varieties and of the lines derived from them ranged from 10.2 to 62.5%, with infection rates below 20% for 11 lines. A line of Bánkúti 1201 origin (BKT9086-95), which had proved tolerant in FHB tests, was crossed with the moderately susceptible wheat variety Mv Magvas. The 219 SSD lines developed from this combination were then tested for Type II resistance (Fig. 2). The infection levels of the lines, parents and control varieties ranged from 5.0 to 72.3%.

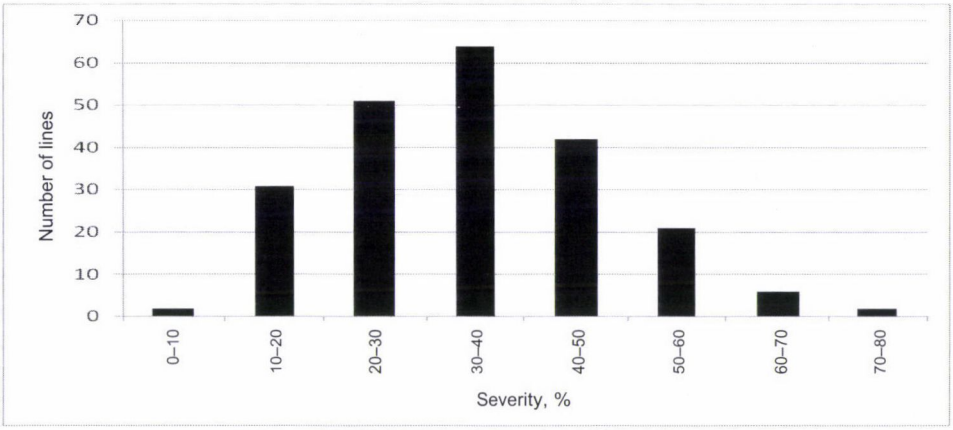

Fig. 2. Distribution of BKT9086-95/Mv Magvas lines according to their type II *Fusarium* head blight resistance, Martonvásár, 2003–2007

Under greenhouse conditions it was found that several varieties and advanced lines bred in Martonvásár had reliable resistance to race 1 of tan spot. Correlation analysis revealed a significantly positive moderate correlation (r = 0.4–0.6) between the greenhouse data (AUDPC values calculated on the basis of lesion type and severity %) and the field data (AUDPC values calculated from the lesion type and severity % on the flag leaf). The extensive analysis of breeding lines could contribute to further improvements in the complex disease resistance of future Martonvásár wheat varieties and to an increase in selection efficiency.

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References

Bai, G. H., Shaner, G. (1994): Scab of wheat: Prospects for control. *Plant Dis.*, **78**, 760–766.

Bizzarri, M. (2009): *Espressione di nuovi geni che conferiscono resistenza ad oidio, ruggine bruna e carie in linee di frumento tenero derivate da ibridazione* Triticum aestivum *L.* × Dasypyrum villosum *(L.) P. Candargy*. MS Thesis, University of Tuscia, Viterbo, Italy.

Bizzarri, M., Vaccino, P., Pasquini, M., Nocente, F., Bianchi, J., Cionini, P. G., Caceres, E., De Pace, C. (2007): The genetics of wheat powdery mildew resistance expressed after the introgression of chromosome 6V in *T. aestivum*. In: *Proceedings of the 51st Italian Society of Agricultural Genetics Annual Congress,* Palazzo dei Congressi, Riva del Garda (TN), 23–26 Sep 2007, Abstr C.37.

Collard, B. C. Y., Mackill, D. J. (2008): Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Phil. Trans. R. Soc. B,* **363**, 557–572.

Cséplő, M., Vida, G., Bakonyi, J., Veisz, O. (2004): Studies on the resistance of wheat genotypes to two different races of *Pyrenophora tritici repentis* (Died.) Drechsler. pp. 185–188. In: Wollmann, J., Grausgruber, H., Ruckenbauer, P. (eds.), *Genetic Variation for Plant Breeding, Proc. of 17th EUCARPIA General Congress,* 8–11 September 2004, Tulln, Austria.

Csősz, M., Kopahnke, D., Nagyhaska, E., Pusztai, I., Mesterházy, Á. (2003): Resistance of winter wheat cultivars against necrotrophic leaf pathogens (2001–2003 Szeged, Hungary and 2003 Aschersleben, Germany). *Proceedings of the 3rd International Plant Protection Symposium at Debrecen University,* 15–16 October, 2003 Debrecen, Hungary, pp. 166–170.

Feuillet, C., Travella, S., Stein, N., Albar, L., Nublat, A., Keller, B. (2003): Map-based isolation of the leaf rust disease resistance gene Lr10 from the hexaploid wheat genome. *Proc. Natl. Acad. Sci. USA,* **100**, 15253–15258.

Lagudah, E. S., McFadden, H., Singh, R. P., Huerta-Espino, H., Bariana, H. S., Spielmeyer, W. (2006): Molecular genetic characterization of the Lr34/Yr18 slow rusting resistance gene region in wheat. *Theor. Appl. Genet.,* **114**, 21–30.

Lamari, L., Bernier, C. C. (1989): Evaluation of wheat lines and cultivars to tan spot *Pyrenophora tritici-repentis* based on lesion type. *Can. J. Plant Pathol.,* **11**, 49–56.

McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C., Appels, R., Xia, X. C. (2010): Catalogue of gene symbols for wheat. In: *Komugi – Integrated wheat science database.* [Available online: http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp. Accessed 16 July 2011]

Mohler, V., Hsam, S. L. K., Zeller, F. J., Wenzel, G. (2001): An STS marker distinguishing the rye-derived powdery mildew resistance alleles at the Pm8/Pm17 locus of common wheat. *Plant Breeding,* **120**, 448–450.

Qiu, J. W., Schürch, A. C., Yahiaoui, N., Dong, L. L., Fan, H. J., Zhang, Z. J., Keller, B., Ling, H. Q. (2007): Physical mapping and identification of a candidate for the leaf rust resistance gene Lr1 of wheat. *Theor. Appl. Genet.,* **115**, 159–168.

Robert, O., Abelard, C., Dedryver, F. (1999): Identification of molecular markers for the detection of the yellow rust resistance gene Yr17 in wheat. *Mol. Breeding,* **5**, 167–175.

Shaner, G., Finney, R. E. (1977): The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology,* **67**, 151–156.

Stubbs, R. W., Prescott, J. M., Saari, E. E., Dubin, H. J. (1986): *Cereal Disease Methodology Manual.* CIMMYT, Mexico, 46 pp.

Szunics, L., Szunics, Lu., Vida, G., Bedő, Z., Svec, M. (2001): Dynamics of changes in the races and virulence of wheat powdery mildew in Hungary between 1971 and 1999. *Euphytica,* **119**, 143–147.

Vida, G., Komáromi, J., Szunics, L., Láng, L., Bedő, Z., Veisz, O. (2007): Búzalisztharmat-virulencia felmérése és szántóföldi rezisztenciavizsgálatok Martonvásáron. (Survey of powdery mildew virulence and field resistance testing in Martonvásár.) *Növénytermelés,* **56**, 3–11.

Winzeler, M., Mesterházy, Á., Park, R. F., Bartos, P., Csősz, M., Goyeau, H., Ittu, M., Jones, E., Löschenberger, F., Manninger, K., Pasquini, M., Richter, K., Rubiales, D., Schachermayr, G., Strzembicka, A., Trottet, M., Unger, O., Vida, G., Walther, U. (2000): Resistance of European winter wheat germplasm to leaf rust. *Agronomie,* **20**, 783–792.

Zadoks, J. C., Chang, T. T., Konzak, C. F. (1974): A decimal code for the growth stages of cereals. *Weed Res.,* **14**, 415–421.

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DISTRIBUTION OF DWARFING GENES (*RHT-B1B* AND *RHT-D1B*) IN MARTONVÁSÁR WHEAT BREEDING MATERIALS

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A total of 266 Martonvásár (Mv) wheat (*Triticum aestivum* L.) accessions, including varieties and advanced lines, were examined using the "Perfect" molecular markers to detect the *Rht-B1b* (formerly *Rht1*) and *Rht-D1b* (formerly *Rht2*) semi-dwarfing genes. The gene *Rht-B1b* was detected in a total of 221 (83.5%) accessions. The *Rht-D1b* allele was found in fewer accessions. Overall 24 genotypes (9%) contained this allele. The analysis of the development date of the genotypes revealed that the introduction of the dwarfing genes into Martonvásár breeding programmes started in the early 1970s, and they were widely utilized from the 1980s. The *Rht-B1b* allele was the main source for reducing plant height, while the *Rht-D1b* allele played only a minor role in the Martonvásár breeding programme.

Characterizing accessions using various molecular markers allows us to create a database offering relevant marker information about genotypes. Such a database could be very helpful for selection, allowing breeders to include varieties giving positive results in specific breeding programmes.

Key words: semi-dwarfing gene, *Rht-B1b, Rht-D1b, Triticum aestivum*

Introduction

The use of dwarfing genes to reduce plant height, increase the harvest index, improve lodging resistance and increase grain yield has been one of the major strategies in developing modern bread wheat *(T. aestivum)* cultivars. To date, twenty-one different dwarfing genes (Rht = reduced height) (McIntosh et al., 1995), each influenced by variations in environmental effects, have been shown to determine the plant height of wheat genotypes. The semi-dwarfing genes in Norin 10 (*Rht-B1b* and *Rht-D1b*) and Akakomugi *(Rht8)* were initially introduced into Italy, the USA and Mexico (CIMMYT) in the first half of the twentieth century, and later spread throughout North and South America, Europe and Asia (Gale and

Youssefian, 1985). The major *Rht* genes are classified into two groups, depending on their response to exogenous gibberellic acid (GA), as GA-insensitive and GA-responsive. The wheat cultivars bred in Hungary were previously examined for the presence or absence of different dwarfing genes by their response to GA3 (Bedő and Balla, 1980). The GA-insensitive semi-dwarfing genes *Rht-B1b (Rht1)* and *Rht-D1b (Rht2)* have been successfully utilized by plant breeders worldwide for more than three decades (Börner et al., 1996). Today more than 70% of modern wheat cultivars contain major dwarfing genes (Evans, 1998). In northwest Europe, the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* are widely used. These genes have comparable mutations in two homoeologous genes on chromosomes 4B and 4D in the hexaploid wheat genome (Börner et al., 1996; McCartney et al., 2005). The homoeologous genes *Rht-B1* and *Rht-D1* were molecularly characterized and both mutations were found to involve single base-pair changes leading to a TAG stop codon shortly after the start of translation (Peng et al., 1999). Furthermore, PCR-based specific markers were developed to discriminate between the dwarf genes *Rht-B1b* and *Rht-D1b* and their wild-type tall alleles *Rht-B1a* and *Rht-D1a* (Ellis et al., 2002).

Materials and methods

In total, 266 Martonvásár wheat accessions were examined in this study. Many of the accessions (181 out of 266) were advanced lines, while the others were varieties. The study includes 11 varieties developed in the 1970s, 11 developed in the 1980s and 31 developed in the 1990s. All the other materials were developed from 2000 to date.

For the molecular study, DNA was extracted from the leaves of wheat seedlings with a QIAcube (Qiagen, Germany) automated workstation using a Qiagen DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The presence or absence of genes *Rht-B1b* and *Rht-D1b* was detected using the molecular markers developed by Ellis et al. (2002). The PCR products were separated on 1.5% agarose gel and visualised by staining with ethidium bromide (Fig. 1).

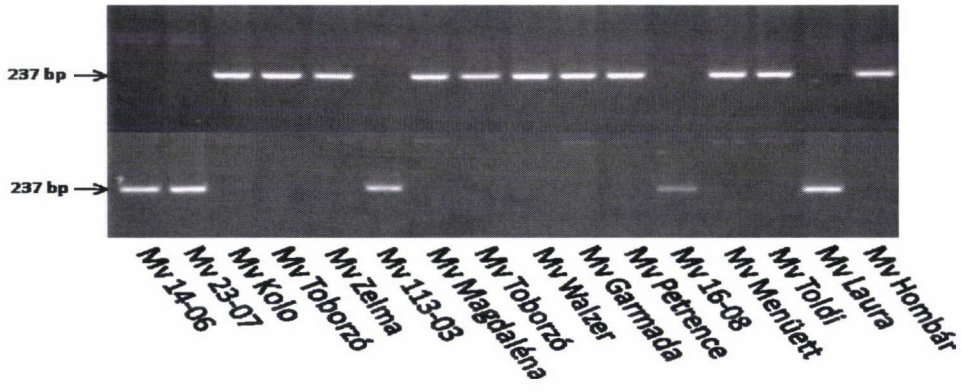

Fig. 1. Detection of the *Rht-B1b* dwarfing allele (upper part) and the wild type (lower part) in some Martonvásár breeding materials using the BF-MR1 and BF-WR1 primer pairs, respectively

Results

The *Rht-B1b* allele was identified in 158 (87.3%) varieties and in 64 (75.3%) advanced lines (Table 1). Interestingly, only one variety carried this gene among those that were developed in the 1970s, while 7 out of 11 varieties carried this gene among those that were developed in the 1980s. Many of the varieties (29 out of 31) that were developed in the 1990s carried this major dwarfing gene. Among the genotypes developed from 2000 onwards, there are only five varieties (5.9%) and 23 advanced lines (12.7%) without the *Rht-B1b* allele.

Table 1
Presence or absence of major dwarfing genes in Martonvásár materials

	No allele		*Rht-B1b*		*Rht-D1b*		*Rht-B1b* and *Rht-D1b*		Total
	No.	%	No.	%	No.	%	No.	%	
Advanced lines	7	3.9	158	87.3	20	11.0	4	2.2	181
Varieties	15	17.6	64	75.3	4	4.7	0	0.0	85
Total	22	8.3	222	83.5	24	9.0	4	1.5	266

The *Rht-D1b* gene was found in fewer accessions. Overall 24 (9%) genotypes (4 varieties and 20 advanced lines) contained this allele. The first appearance of this gene was detected in advanced lines crossed in 1996 and was found in the variety Mv Laura, registered in 2007. In total, four advanced lines (1.5%) and no varieties contained both alleles. Neither of the two dwarfing genes was identified in 22 (8.3%) genotypes.

All the varieties used in this study are listed according to their developmental year in Table 2.

Table 2
List of varieties indicating the presence or absence of *Rht* genes

Variety	*RhtB1b*	*RhtD1b*	Plant height (2010), (cm)	Variety	*RhtB1b*	*RhtD1b*	Plant height (2010), (cm)
Martonvásári 2	0	0	85.0	Mv Szigma	1	0	85.0
Martonvásári 3	0	0	85.0	Mv Magdaléna	1	0	77.0
Martonvásári 4	0	0	89.0	Mv Madrigál	1	0	89.0
Martonvásári 5	0	0	80.0	Mv Matador	1	0	84.0
Martonvásári 7	0	0	78.0	Mv Magvas	1	0	85.0
Martonvásári 8	0	0	79.0	Mv Mezőföld	1	0	81.5
Martonvásári 9	1	0	84.0	Mv Summa	1	0	79.0
Martonvásári 10	0	0	76.0	Mv Tamara	1	0	76.0
Martonvásári 11	0	0	80.0	Mv Martina	1	0	83.0
Martonvásári 12	0	0	65.0	Mv Kucsma	1	0	77.0
Martonvásári 13	0	0	74.0	Mv Csárdás	1	0	87.0

Table 2 (cont.)

Variety	*RhtB1b*	*RhtD1b*	Plant height (2010), (cm)	Variety	*RhtB1b*	*RhtD1b*	Plant height (2010), (cm)
Martonvásári 14	1	0	76.0	Mv Palotás	1	0	76.0
Martonvásári 15	1	0	80.0	Mv Prizma	1	0	76.0
Martonvásári 16	0	0	79.0	Mv Matild	1	0	78.0
Martonvásári 17	1	0	77.0	Mv Mariska	1	0	77.0
Martonvásári 18	0	0	78.0	Mv Emese	1	0	81.0
Martonvásári 19	0	0	83.0	Mv Dalma	1	0	71.0
Martonvásári 20	1	0	85.0	Mv Amanda	1	0	67.0
Martonvásári 21	1	0	78.0	Mv Marsall	1	0	74.0
Martonvásári 22	1	0	78.0	Mv Mambó	1	0	74.5
Martonvásári 23	0	0	81.0	Mv Panna	1	0	79.0
Martonvásári 24	1	0	80.0	Mv Verbunkos	1	0	75.0
Martonvásári 25	1	0	62.0	Mv Vekni	1	0	81.0
Mv Pálma	0	0	75.0	Mv Ködmön	1	0	86.0
Mv Magma	0	0	61.0	Mv Süveges	1	0	82.0
Mv Koma	1	0	84.0	Mv Suba	1	0	83.0
Mv Optima	1	0	84.0	Mv Garmada	1	0	80.0
Mv Irma	1	0	86.0	Mv Béres	1	0	80.0
Mv Emma	1	0	83.0	Mv Piroska	1	0	88.0
Mv Vilma	1	0	84.0	Mv Toborzó	1	0	79.0

Variety	*RhtB1b*	*RhtD1b*	Plant height (2010), (cm)
Mv Walzer	1	0	85.0
Mv Mazurka	1	0	81.0
Mv Matyó	1	0	75.0
Fatima 2	1	0	74.0
Mv Táltos	1	0	77.0
Mv Regiment	1	0	76.0
Mv Kemence	1	0	76.0
Mv Hombár	1	0	73.5
Mv Gorsium	1	0	58.0
Mv Kolo	1	0	84.5
Mv Zelma	1	0	85.0
Mv Laura	0	1	85.0
Mv Lucilla	1	0	75.0
Mv Toldi	1	0	85.0
Mv Bodri	0	1	74.0
Mv Menüett	1	0	87.0
Mv Petrence	1	0	75.0
Mv Kolompos	1	0	86.0
Mv Karizma	0	1	91.0
Mv Apród	1	0	78.0
Mv Tallér	1	0	76.0
Mv Kikelet	1	0	90.0
Mv Karéj	1	0	76.0
Mv Sobri	0	1	87.0
Mv Lepény	0	0	76.0

Discussion

According to the results, either the *Rht-B1b* or the *RhtD1b* allele can be found in 90.1% of the Martonvásár wheat breeding materials investigated. A similar result was published by Borlaug (1968), who presented data showing that 90% of the semi-dwarf genotypes worldwide carry one of the major factors. However, according to the study of Knopf et al. (2008) the two semi-dwarfing alleles were present in only 43.6% of the varieties in Germany.

By focusing on the development date of the investigated genotypes compared with the presence of the dwarfing genes, it can be concluded that the introduction of the dwarfing genes into breeding programmes in Martonvásár happened in the early 1970s. The *Rht-B1b* allele was the main source for reducing plant height in Martonvásár, together with the GA-sensitive *Rht8* allele (data not shown). The *Rht-D1b* allele played only a minor role in the reduction of plant height in the Martonvásár breeding programme. Interestingly, in China and Germany the frequency of the *Rht-D1b* allele was higher than that of the *Rht-B1b* allele (Zhang et al., 2006; Knopf et al., 2008).

The semi-dwarfing genes *Rht-B1b* and *Rht-D1b* were introduced into Hungary with cultivars originating from South America and Western Europe (Bognár et al., 2007). Pedigree analysis revealed (data not shown) that the spread of the *Rht-B1b* allele was enhanced by using the Fatima-2 variety and the MvMa breeding line in Martonvásár.

The *Rht-D1b* allele was found in four varieties, including Mv Laura, which has Ukrainka-Odesskaya, a variety having the *Rht-D1b* allele, in its pedigree. In addition, other sources of dwarfism were included in Martonvásár breeding programmes, such as the varieties Tom Thumb (*Rht-B1b*) and Olesen's Dwarf (*Rht-B1b* and *Rht-D1b*).

The lack of the two alleles in 22 genotypes can be explained by the fact that 21 genes with major effects in reducing plant height have been identified in wheat and assigned *Rht* designations (McIntosh et al., 1995). Therefore, other plant height-reducing genes may also be contained by these accessions.

This study demonstrated the presence of the semi-dwarfing genes *Rht-B1b* and *Rht-D1b* in the Martonvásár breeding programme. The molecular markers used in this study made it possible to characterize the genotypes that played a major role in the past and those currently used. Putting this information into databases could be very helpful for selection, allowing breeders to include varieties with positive results in specific breeding programmes. In the Agricultural Research Institute (HAS) researchers have put much effort into creating such a database with relevant marker information on parental lines and breeding materials in order to facilitate the breeding of new varieties with better adaptability to a changing environment.

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References

Bedő, Z., Balla, L. (1980): Effect of GA3 treatment during vernalisation on various types of winter wheat. *Cereal Res. Commun.,* **8**, 545–550.

Bognár, Z., Láng, L., Bedő, Z. (2007): Effects of environment on the plant height of wheat germplasm. *Cereal Res. Commun.,* **35**, 281–284.

Borlaug, N. E. (1968): Wheat breeding and its impact on world food supply. pp. 5–15. In: Finlay, E. W., Sheperd, K. W. (eds.), *Proceedings of the 3rd International Wheat Genetics Symposium.* Australian Academy of Science, Canberra.

Börner, A., Plaschke, J., Korzun, V., Worland, A. J. (1996): The relationships between the dwarfing genes of wheat and rye. *Euphytica,* **89**, 69–75.

Ellis, M. H., Spielmeyer, W., Gale, K. R., Rebetzke, G. J., Richards, R. A. (2002): "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theor. Appl. Genet.,* **105**, 1038–1042.

Evans, L. T. (1998): *Feeding the Ten Billion: Plants and Population Growth.* Cambridge University Press, Cambridge, pp. 133–150.

Gale, M. D., Youssefian, S. (1985): Dwarfing genes in wheat. pp. 1–35. In: Russed, G. E. (ed.), *Progress in Plant Breeding* Vol 1. Butterworths, London.

Knopf, C., Becker, H., Ebmeyer, E., Korzun, V. (2008): Occurrence of three dwarfing *Rht* genes in German winter wheat varieties. *Cereal Res. Commun.,* **36**, 553–560.

McCartney, C. A., Somers, D. J., Humphreys, D. G., Lukow, O., Ames, N., Noll, J., Cloutier, S., McCallum, B. D. (2005): Mapping quantitative trait loci controlling agronomic traits in the spring wheat cross RL44523'AC Domain'. *Genome,* **48**, 870–883.

McIntosh, R. A., Hart, G. E., Gale, M. D. (1995): Catalogue of gene symbols for wheat. pp. 1333–1500. In: Li, Z. S., Xin, Z. Y. (eds.), *Proc of 8th Int. Wheat Genet. Symp.* China Agricultural Scientech Press, Beijing.

Peng, J. R., Richards, D. E., Hartley, N. H., Murphy, G. P., Devos, K. M., Flintham, J. E., Beales, J., Fish, L. J., Worland, A. J., Pelica, F., Sudhakar, D., Christou, P., Snape, J. W., Gale, M. D., Harberd, N. P. (1999): "Green revolution" genes encode mutant gibberellin response modulators. *Nature,* **400**, 256–261.

Zhang, X., Yang, S., Zhou, Y., He, Z., Xia, X. (2006): Distribution of the *Rht-B1b, Rht-D1b* and *Rht8* reduced height genes in autumn-sown Chinese wheats detected by molecular markers. *Euphytica,* **152**, 109–116.

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COMPOSITION OF *FUSARIUM* SPECIES CAUSING NATURAL SPIKE INFECTION IN WHEAT

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Numerous *Fusarium* species have been associated with the *Fusarium* head blight (FHB) disease of wheat, barley and other small-grain cereals, reducing worldwide cereal crop yields and, as a consequence of their mycotoxin production in the cereal grain, having an impact on both human and animal health.

The year 2010 was extremely favourable for *Fusarium* head blight pathogens. Over a hundred symptomatic wheat heads were collected from various locations in Hungary. The aim was to determine the diversity of the *Fusarium* species infecting winter wheat ears. A total of 86 *Fusarium* spp. were morphologically identified from diseased kernels. *F. sambucinum* was found to be present in two of the Martonvásár samples. This pathogen had only previously been detected extremely sporadically. The species *F. culmorum* and *F. verticillioides* were found at a much lower rate than expected, while none of the isolates were identified as *F. poae*. On the basis of the results, 95% of the isolates belonged to the *Fusarium graminearum* species complex.

Key words: *Fusarium* head blight, classical taxonomy

Introduction

Climate change may have an influence on plant pathogen distribution, so breeders must monitor these changes and prepare to face new challenges. This is especially true in the case of *Fusarium* species, since these pathogens attack many crops, including wheat, the most important cereal species in Hungary.

Various climate models predict that the annual mean temperature in the Carpathian Basin will rise by 2.5–4.8°C, depending on the model (Bartholy et al., 2007). The greatest increase in temperature can be expected in the summer months (4.0–4.8°C) and the smallest in the spring (2.5–3.1°C). The annual precipitation sum is not expected to change significantly in this region (Bartholy et al.,

2008), but this is not true of the seasonal precipitation sums. Summer precipitation is likely to decrease, while a slight increase is predicted in spring by all the models.

To avoid heat stress at anthesis, it is likely that wheat varieties with shorter vegetation periods will be more widely grown. However, the higher mean temperature in spring and the larger rainfall sum could have a negative effect, as they could prove favourable for the diseases to which the crop is susceptible during flowering, such as *Fusarium* head blight (FHB).

The fungal cereal disesase complex *Fusarium* head blight, also called *Fusarium* scab of small grain cereals, is caused by a complex of as many as 17 different species in the genera *Fusarium* and *Microdochium,* of which *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* and *Microdochium nivale* are predominant (Leonard and Bushnell, 2003; Brennan et al., 2007). Airborne spores released from crop residues are deposited on or inside florets, where they germinate and initiate infection. Anthesis is therefore the most vulnerable growth stage for establishing successful infection.

FHB is among the most destructive and economically important diseases of wheat worldwide and has been extensively studied over the last decades (Wagacha and Muthomi, 2007; Xu and Nicholson, 2009). Epidemics caused by FHB pathogens result in severe yield losses and a decline in cereal quality. Furthermore, infection by these pathogens leads to the contamination of the grain and straw with a wide array of mycotoxins. These fungal metabolites pose serious threats to human and animal health (Wu and Munkvold, 2008), while trichothecenes inhibit eukaryotic protein synthesis and modify the immune function (Pestska and Smolinski, 2005).

Although resistance to FHB is not species-specific, it is nevertheless important to determine which of the naturally occurring pathogens are dominant, so as to ensure that the most frequently occurring species are used for artificial inoculation in the course of resistance breeding and that a rapid response can be made to possible changes in aggressiveness.

Materials and methods

Disease assessment and field sampling

Fields in four locations (representing the main wheat growing regions in Hungary – Martonvásár, Kisújszállás, Lippó, Szeged) were evaluated for the presence of *Fusarium* spp. symptoms between 1st May and 10th July 2010.

In order to obtain a representative image of the FHB population at each location symptomatic heads were harvested randomly during the disease assessment period. Two distinctly symptomatic seeds were isolated from each ear.

Standard media

Potato dextrose agar (PDA) was used for observing colony morphology. The microscopic features used for species identification by Nelson et al. (1983) are based on growth on carnation leaf agar (CLA). This was replaced by synthetic nutrient agar (SNA), as *Fusarium* species also produce typical, morphologically identical macroconidia (in sporodochia) on this medium.

Naturally infected kernels were sterilised in 70% ethanol and then placed on SNA. When observing fusoid macroconidia, single spores were transferred onto PDA and SNA plates.

Growth conditions

The production of macroconidia (in sporodochia) and pigmentation are favoured by light that includes ultraviolet radiation. All the *Fusarium* species occurring on cereals favour a temperature range of 20–21°C. Dishes containing PDA were placed under diffuse daylight, while one set of SNA plates was kept under black UV light and the other in the dark.

Identification

The genus *Fusarium* was set up by Link (1809) for species with fusiform, non-septate spores. Since the development of pure culture methods for *Fusarium* identification, the presence of fusoid macroconidia with a foot-shaped basal cell has been accepted as the most definitive characteristic of the genus. The species are arranged in sections, which are based solely on similar morphological characters (shape of the macroconidium, shape of the basal cell of the macroconidium, presence or absence of microconidia, shape of the microconidium, presence or absence of chlamydospores, and location of chlamydospores).

Morphological identification was based on Nelson et al. (1983) and Leslie and Summerell (2006) (Fig. 1).

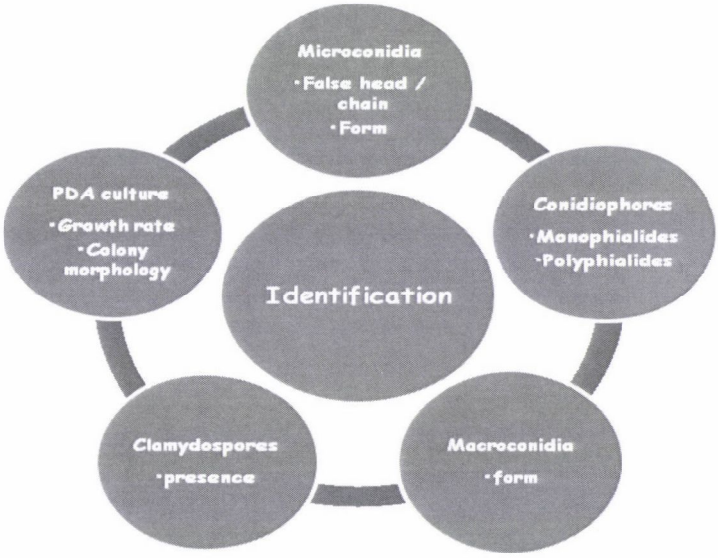

Fig. 1. Morphological markers

Microconidial production and conidigenous cells were viewed in 4–7-day-old cultures. The way they were formed (false heads and/or chains), their shape and the nature of the conidigenous cells (mono- or polyphialides) are important characteristics. Macroconidia were examined in 10–14-day-old cultures and the general shape was noted (this is a key marker for species in the sections Arthrosporiella, Discolor, Gibbosum and Roseum). Chlamydospores tended to form in older cultures (3–4 weeks old); formation may vary between isolates from the same species and even between successive cultures of a single isolate. Thus, while their presence was a useful criterion, their absence was not. The growth rate and colony morphology were observed on PDA plates.

Results and discussion

In order to determine the pathogen composition, 100 symptomatic kernels were selected for the morphological identification of *Fusarium* spp. (Table 1). It proved possible to identify the species causing the symptoms in 84% of the samples, but in 16 cases the presence of *Fusarium* species was not detected. Of the species identified, 95% belonged to the *Fusarium graminearum* species complex. The presence of *F. sambucinum,* a pathogen previously found extremely sporadically, was detected in two of the Martonvásár samples, while *F. culmorum* and *F. verticillioides* were isolated in a far smaller ratio than expected. Among the samples from Kisújszállás, the pathogen was only identified in two cases, while it proved impossible to determine whether the mycelia present on the other 10 wheat kernels originated from pathogens belonging to the *Fusarium* genus.

Table 1
Origin and number of *Fusarium* spp. isolates

	Origin			
Species	Martonvásár	Kisújszállás	Lippó	Szeged
F. graminearum	49	2	10	19
F. culmorum	1			
F. sambucinum	2			
F. verticilloides				1
Not identified		10	4	2
	84 out of 100 isolates were identified			

Fusarium graminearum particularly likes higher temperatures, whereas *F. culmorum, F. poae, F. avenaceum* and *M. nivale* tend to dominate in cooler regions such as Scandinavia and the UK (Doohan et al., 2003; Lukanowski et al., 2008). The two main DON-producing species, *F. graminearum* and *F. culmorum,* have slightly different temeperature optima for growth, 24–28°C for *F. gramiearum* and 20–25°C for *F. culmorum* (Brennan et al., 2003; Doohan et al., 2003). This small difference may explain why *F. graminearum* predominates in regions with relatively hot summers, such as the USA, Canada, Australia and parts of continental Europe. In Hungary, *F. poae* was reported to be a predominant

component of the FHB disease complex (Xu et al., 2008), but the present results indicate that the *F. graminearum* species complex was dominant in 2010.

It has recently been reported that highly toxigenic *F. graminearum* populations are spreading widely in North America (Ward et al., 2008). The present results confirm that it is worth monitoring not only the species composition of the pathogens causing FHB, but also changes in variability within the species, so that the results can be used in wheat breeding programmes.

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References

Bartholy, J., Pongrácz, R., Gelybó, G. (2007): Regional climate change expected in Hungary for 2071–2100. *Applied Ecology and Environmental Research,* **5**, 1–17.

Bartholy, J., Pongrácz, R., Gelybó, G., Szabó, P. (2008): Analysis of expected climate change in the Carpathian Basin using the PRUDENCE results. *Időjárás,* **112**, 249–264.

Brennan, J. M., Fagan, B., van Maanen, A., Cooke, B. M., Doohan, F. M. (2003): Studies on *in vitro* growth and pathogenicity of European *Fusarium* fungi. *Eur. J. Plant Pathol.,* **109**, 577–587.

Brennan, J. M., Leonard, G., Fagan, B., Cooke, B. M., Ritieni, A., Ferracane, R. (2007): Comparison of commercial European wheat cultivars to *Fusarium* infection of head and seedling disease. *Plant Pathol.,* **56**, 55–64.

Doohan, F. M., Brennan, J., Cooke, B. M. (2003): Influence of climatic factors on *Fusarium* species pathogenic to cereals. *Eur. J. Plant Pathol.,* **109**, 755–768.

Leonard, K., Bushnell, W. (2003): *Fusarium Head Blight of Wheat and Barley*. APS Press, St. Paul, MN, pp. 44–83.

Leslie, J. F., Summerell, A. B. (2006): *The Fusarium Laboratory Manual*. Blackwell Publishing Professional, Ames, IA, 388 pp.

Link, H. F. (1809): Observationes in ordines plantarum naturales. Dissertatio I. *Mag. Ges. Naturf. Freunde*, Berlin, **3**, 3–42.

Lukanowski, A., Lenc, L., Sadowski, C. (2008): First report on the occurrence on *Fusarium langsethiae* isolated from wheat kernels in Poland. *Plant Dis.,* **92**, 488–490.

Nelson, P. E., Toussoun, T. A., Marasas, W. F. O. (1983): *Fusarium Species. An Illustrated Manual for Identification*. Pennsylvania State University Press, University Park, PA, 193 pp.

Pestska, J. J., Smolinski, A. T. (2005): Deoxynivalenol: toxicology and potential effects on humans. *J. Toxicol. Environ. Health B Crit. Rev.,* **8**, 39–69.

Wagacha, J. M., Muthomi, J. W. (2007): *Fusarium culmorum:* Infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. Review. *Crop Protect.,* **26**, 877–885.

Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D. E., Gilbert, J., Geiser, D. M., Nowicki, T. W. (2008): An adaptive evolutionary shift in

Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. *Fungal Genet. Biol.*, **45**, 437–484.

Wu, F., Munkvold, G. P. (2008): Mycotoxins in ethanol co-products: Modelling economic impacts on the livestock industry and management strategies. *J. Agr. Food. Chem.*, **56**, 3900–3911.

Xu, X., Nicholson, P. (2009): Community ecology of fungal pathogens causing wheat head blight. *Annu. Rev. Phytopathol.*, **47**, 83–103.

Xu, X., Parry, D. W., Nicholson, P., Thomsett, M. A., Simpson, D., Edwards, S. G. (2008): Within-field variability of *Fusarium* head blight pathogens and their associated mycotoxins. *Eur. J. Plant. Pathol.*, **120**, 21–34.

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SUSTAINABLE WHEAT PRODUCTION IN A CHANGING CLIMATE

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The dependence of winter wheat production on spring temperature and precipitation was analysed on the basis of historical meteorological data. Production was found to be a linear function of temperature, so increasing temperatures resulted in lower yields. The dependence of yield on precipitation could be described with a quadratic function, and the yield decreased above the optimal precipitation amount. The results of regression analysis are presented using 30-year data for Fejér County. Simulation modelling was used to analyse the suitability of future climates for growing winter wheat in Hungary. The locations chosen were heterogeneous in terms of meteorological conditions, but were all relatively flat and of great importance for Hungarian winter wheat production: Győr-Moson-Sopron County in W. Hungary, which is well supplied with precipitation, Hajdú-Bihar County in the east, where the weather is warmer and drier, and Pest County in the middle of the country. Evaluations were made using the Ceres-Wheat model and a modified version of AFRCWHEAT2. An analysis of the simulation results revealed that agricultural productivity is close to the upper limit of what can be achieved using conventional methods, so decreased yields and an increase in production risks are probable in the future in all three regions.

Key words: wheat production, temperature, precipitation, simulation modelling

Introduction

Hungary is located at the junction of the Atlantic, Mediterranean and Continental climate zones, all three of which influence weather conditions, which are consequently extremely variable. Despite the frequency of extreme weather events, however, the conditions are very favourable for agricultural production (Harnos, 1996).

Climate change scenarios for Hungary generally predict a mean rise of 1°C for the spring and autumn months and 2–4°C in the winter and summer months up to the middle of the century, rising to 3–5°C by the end of the century. Changes in rainfall sums may differ in the western and eastern parts of the country. They are likely to increase in the winter months, while rainfall in summer may drop by 20–30% in the eastern regions (Bartholy et al., 2007). Among the agricultural effects of global climate change, variability in the quantity of rainfall during the vegetation period is likely to have the most limiting effect on yield reliability.

In the present work a long-term data series was used to examine the closeness of the correlation between winter wheat yields and the temperature and rainfall sums in spring, and simulation models were used to describe what changes can be expected under the climatic conditions of the future.

Materials and methods

Regression analysis was performed on the mean winter wheat yields recorded in Fejér County over the period 1981–2008 and on daily temperature and rainfall data.

The simulation was run (Harnos and Erdélyi, 2008) using a modified version of the previously tested and validated AFRCWHEAT2 model (Porter, 1993), designated as the AF2MOD model, and the Ceres-Wheat model (Ritchie and Otter, 1985). These simulation models have already been successfully tested and validated using long-term climatic and yield datasets (Harnos and Erdélyi, 2008).

The effects of climate change were examined on the winter wheat yields obtained in three geographically and meteorologically diverse regions of Hungary: Győr-Moson-Sopron County, a wetter region in the west, Pest County, representative of the central part of the country, and Hajdú-Bihar County, an important agricultural area in the warmer, drier region of Eastern Hungary. In this way the effects of climate change could be examined at approximately the same latitude from west to east.

The climate change data series used for the simulations were downloaded from the homepage of the Department of Meteorology, Eötvös Loránd University, Budapest for the 2070–2100 period (Bartholy et al., 2007). Two data series were used, both of which included a control data series representing the last third of the 20th century. One of the data series was compiled by the Hadley Centre (HC) and the other by the Max Planck Institute (MPI). The control runs (1960–1990) and the A2 scenario (a composite of 16 model runs) were used from both data series. The given datasets contain daily minimum and maximum temperatures and daily precipitation. Other climatic data required by the models were calculated as described in Harnos (2003) and global radiation data were estimated with the method of Ritchie and Fodor using the 4M model (Fodor et al., 2000).

The means of the results obtained by running the control and scenario data series were compared using the *t*-test and the deviations using the F test.

Results and discussion

Among the meteorological factors, crop production is influenced to the greatest extent by temperature and rainfall. The dependence of the winter wheat yield on weather conditions is illustrated in Figure 1, where the mean yields re-

corded in Fejér County over a period of nearly 30 years are plotted against temperature and rainfall data.

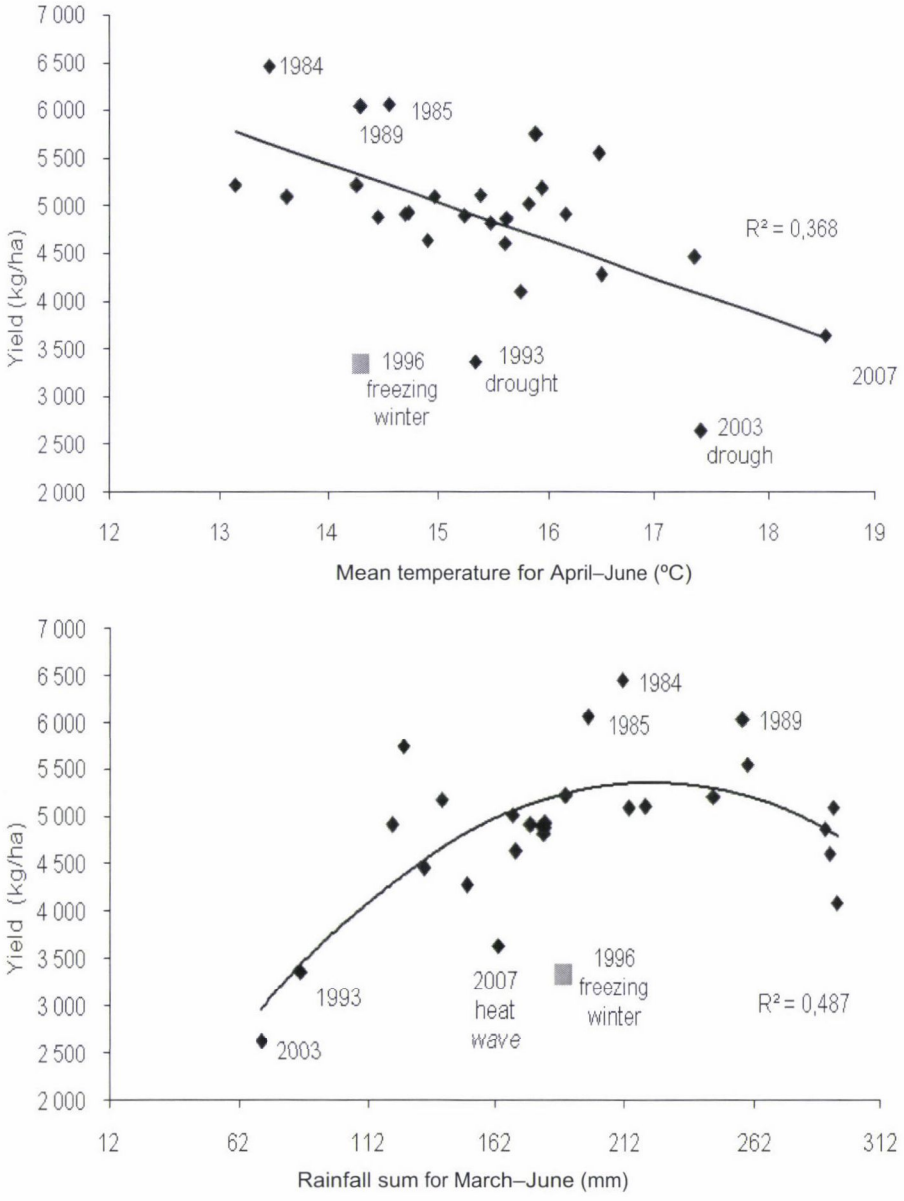

Fig. 1. Winter wheat yield averages in Fejér County during the 1981–2008 period as a function of mean temperature and rainfall quantities in spring. Years with exceptional yield averages are highlighted. The continuous lines represent curves fitted to the points: with the exception of 1996, the winter wheat yields exhibited a linear correlation with temperature ($R^2 = 0.36$) and a quadratic relationship with rainfall quantity ($R^2 = 0.48$)

The data indicate that a third of the winter wheat yield depended on the mean temperature from April to June and half on the rainfall quantity from March to June. Extreme weather conditions intensify each other's effects, in both a positive and a negative direction. In 1984, 1985 and 1989 favourable temperatures combined with relatively high rainfall sums resulted in high yields, while in 2003 the exceptional lack of rainfall combined with high mean temperatures caused considerable yield losses. A very hot spring and summer (2007), rainfall deficiency (1993) or a very cold winter (1996) may in themselves result in yield declines.

In respect to temperature and precipitation for the period 2070–2100, climate change scenarios predict the smallest changes for the western part of Hungary and the most drastic changes in the eastern part. The average expected changes can be seen in Table 1.

Table 1

Changes in mean, maximum and minimum temperatures and annual precipitation in Hungary between 2070 and 2100, based on climate change scenario A (Bartholy et al., 2007)

A2 scenario	Spring	Summer	Autumn	Winter
Temperature mean (°C)	2.9–3.2	4.5–5.1	4.1–4.3	3.7–4.3
Maximum (°C)	2.8–3.3	4.9–5.3	4.3–4.6	3.7–4.2
Minimum (°C)	3.0–3.2	4.2–4.8	4.0–4.2	3.8–4.6
Precipitation (%)	0 – (+10)	(–24) – (–33)	(–3) – (–10)	(+23) – (+37)

The use of simulation models demonstrated that winter wheat yields are likely to decrease in the second half of the century, assuming the present growing conditions (technology and varieties), compared with the present situation, taken as the control (Fig. 2). In terms of yield, the HC scenario of the AF2MOD model did not predict reductions in Pest County, but in all the other cases a drop in grain yield was predicted, which was significant for both climate scenarios in Hajdú-Bihar County and for the MPI scenario in Győr-Moson-Sopron County (Fig. 2). The Ceres-Wheat model simulated significant yield losses for both the climate scenarios. The risk of yield losses rose significantly in Pest and Hajdú-Bihar Counties compared with the control.

Conclusions

The success of agricultural production is reduced to the greatest extent by unfavourable environmental effects, the most important of which is water deficit. The negative changes predicted in the quantity and distribution of rainfall serve to enhance the outstanding significance of the water reserves stored in the soil and available to plants (Várallyay, 2008).

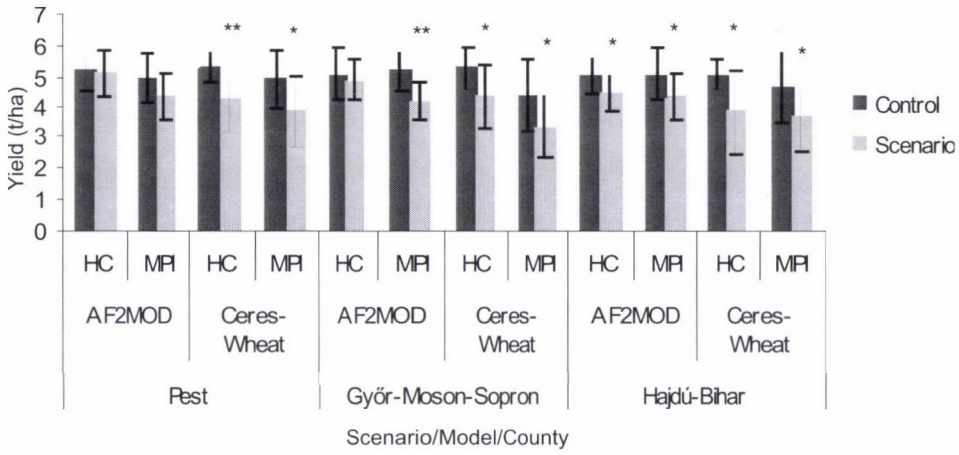

Fig. 2. Mean values and standard deviation of the wheat grain yields simulated by the Ceres-Wheat and AF2MOD models using the HC and MPI climate scenarios

*, **: Differences significant from the control at the 5% and 1% probability levels, respectively.

Unfavourable economic effects may be modified by the increasing concentration of atmospheric CO_2. Differences can be observed in the ability of cereal genotypes to exploit surplus CO_2, primarily to increase the grain yield, so it may be possible to select genotypes capable of adapting efficiently to altered conditions (Varga et al., 2009).

The greatest variability can be detected between technological factors, indicating that the optimum balance between complex interactive effects can only be achieved with great expertise and care. If soil and climatic conditions or the production technology are not of a very high standard, it is not advisable to choose varieties capable of record yields. Varieties with good adaptability are able to produce good yields even if their requirements are not fully met. Yield reliability can be improved by growing varieties with satisfactory adaptability, whose yields exhibit less fluctuation. The primary task facing today's breeders is not to increase yields, but to improve yield quality and reliability and to develop varieties resistant to extreme weather events.

To achieve adaptability to climate change it is thus necessary for experts in various fields to promote higher, more stable yields by developing drought- and heat-resistant varieties, by choosing optimum sowing dates, and by applying water-saving technologies and optimum tillage, and to investigate ways in which carbon dioxide can be more efficiently exploited.

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References

Bartholy, J., Pongrácz, R., Gelybó, G. (2007): Regional climate change expected in Hungary for 2071–2100. *Appl. Ecol. Envir. Res.*, **5**, 1–17.

Fodor, N., Kovács, G. J., Ritchie, J. T. (2000): *A New Solar Radiation Generator for Hungary.* Poster. ASA-CSA-SSSA, Annual Meetings. November 5–9, 2000, Minneapolis, MN, Abstract, p. 23.

Harnos, N. (2003): A klímaváltozás hatásának szimulációs vizsgálata őszi búza produkciójára. (Simulation of the effect of climate change on the yields of winter wheat.) *"AGRO-21" Füzetek*, **31**, 56–73.

Harnos, N., Erdélyi, É. (2008): Alkalmazkodási stratégiák őszi búza termelékenységének fenntartásához szimulációs modellek használatával. (Adaptation strategies for sustaining the productivity of winter wheat using simulation models.) pp. 309–328. In: Harnos, Z., Csete, L. (eds.), *Klímaváltozás: környezet-kockázat-társadalom*. Szaktudás Kiadó Ház, Budapest.

Harnos, Z. (1996): Modelling crop response in Hungary. In: Harrison, P. A., Butterfield, R. E., Cowning, T. E. (eds.), *Climate Change, Climatic Variability and Agriculture in Europe*. Annual Report. Environmental Change Unit, University of Oxford, pp. 179–189.

Porter, J. R. (1993): AFRCWHEAT2 A model of the growth and development of wheat incorporating responses to water and nitrogen. *Eur. J. Agr.*, **2**, 69–82.

Ritchie, J. T., Otter, S. (1985): *Description and Performance of CERES-Wheat: A User-oriented Wheat Yield Model.* US Dept. Agric., ARS, **38**, 159–175.

Várallyay, G. (2008): Extreme soil moisture regime as limiting factor of the plants' water uptake. *Cereal Res. Commun.*, **36**, 3–6.

Varga, B., Bencze, S., Veisz, O. (2009): A szárazság és az emelt légköri CO_2 hatása az őszi búza produktivitására. (Effect of drought and elevated atmospheric CO_2 on the productivity of winter wheat.) pp. 517–521. In: Veisz, O. (ed.), *Hagyomány és haladás a növénynemesítésben.* XV. Növénynemesítési napok (2009. III. 17.), Budapest.

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DEVELOPMENT OF SYNTHETIC AMPHIPLOIDS BASED ON *TRITICUM TURGIDUM* × *T. MONOCOCCUM* CROSSES TO IMPROVE THE ADAPTABILITY OF CEREALS

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Cultivated einkorn (*Triticum monococcum* L. ssp. *monococcum*) is an excellent source of resistance against several wheat diseases and quality parameters. Semi-dwarf einkorn lines with good crossability were identified in order to produce *Triticum turgidum* × *T. monococcum* synthetic amphiploids. Two combinations were used to develop the amphiploids: durum × einkorn and emmer × einkorn.

After the genome duplication of F_1 seeds, highly fertile amphiploids were developed. The A^uBA^m genome structure of the progenies was confirmed by genomic *in situ* hybridization (GISH).

Lines derived from durum × einkorn and emmer × einkorn crosses were studied for agronomic performance, disease resistance and genetic variability. Both amphiploid combinations showed excellent resistance against certain wheat diseases (leaf rust, powdery mildew), but not against fusarium. The durum-based synthetic amphiploid lines showed a higher level of phenotypic diversity. The newly produced *T. turgidum* × *T. monococcum* synthetic hexaploids are promising genetic resources for wheat breeding. Selected durum × einkorn lines are currently used in bread wheat improvement to transfer the useful properties of einkorn into cultivated hexaploid wheat via 'bridge-crossing'.

Key words: *Triticum monococcum* L. ssp. *monococcum, Triticum turgidum* L., synthetic amphiploid, crossability, interspecific hybridization

Introduction

Amphiploids are interspecific hybrids having a diploid set of chromosomes from each parental species. Artificial or synthetic amphiploids play an important role both in plant improvement and in evolutionary studies. The production of synthetic amphiploids is an effective and rapid way of introgressing desirable traits from related species into cultivated wheats (Goncharov et al., 2007). Among

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the different amphiploids, the hexaploid wheat amphiploids have great importance in breeding, because they could be used in wheat breeding as a bridge material. One of the best known artificial amphiploids is synthetic hexaploid wheat (AABBDD, 2n = 6x = 42), derived from the artificial synthesis of hexaploid wheat using various *Triticum turgidum* and *Aegilops tauschii* accessions (Mujeeb-Kazi et al., 1996).

As an alternative to the widely used synthetic hexaploid wheat, several *T. turgidum* × *T. monococcum* ($A^uA^uBBA^mA^m$, 2n = 6x = 42) synthetic amphiploids have also been produced in order to combine the outstanding disease resistance of einkorn with the high productivity of tetraploid wheat species (Gill et al., 1988; Mujeeb-Kazi and Hettel, 1995; Plamenov et al., 2009). In most cases, these amphiploids contain wild einkorn (*T. monococcum* ssp. *aegilopoides,* A^bA^b), which has better crossability than that of cultivated einkorn, which is agronomically more valuable but less crossable (The and Baker, 1975).

Among the *Triticeae*, einkorn wheat (*T. monococcum* L. ssp. *monococcum,* 2n = 2x = 14, A^mA^m) is thought to be one of the most valuable sources of resistance genes for cereal breeding (Monneveux et al., 2000). Several cultivated einkorn genotypes have good agronomic performance; some of them show excellent winter hardiness, drought tolerance, allelopathy and straw strength. Moreover, their high tocol and carotenoid contents make them promising sources for functional food production (Brandolini et al., 2008). Unfortunately, the application of cultivated einkorn in wheat breeding programmes is greatly limited by its poor crossability with wheat.

The aim of the present work was to develop durum × einkorn and emmer × einkorn synthetic amphiploids, as a new crop with high chromosome stability and good agronomic performance. After cytomolecular genome analysis, the agronomic characterization of the new amphiploids was a further goal.

Materials and methods

Plant material

Two tetraploid *Triticum* accessions were used to examine their crossability with diploid, cultivated einkorn (*T. monococcum* L. ssp. *monococcum*). The female partners were tetraploid durum (*T. turgidum* L. ssp. *durum,* MVTD14-04) and emmer wheat (*T. turgidum* L. ssp. *dicoccon,* Mv Hegyes). The pollinators were five traditional einkorn accessions (ID140, Mv Alkor, MVGB361, MVGB747 and G11) and five semi-dwarf einkorn breeding lines (1T-1, 2T-1, 3T-1, 4T-1 and 3T-3). Crossability was expressed as the ratio of the number of seeds set to the total number of flowers pollinated. The crosses were made under field conditions in the years 2006 and 2007.

Genome duplication

The F_1 hybrids (2n = 3x = 21; A^uBA^m) were cytologically confirmed. The genome duplication of F_1 hybrid seedlings with 3–5 tillers was achieved by colchicine treatment (0.04%) as described by Barnabás et al. (1991).

Genomic in situ *hybridization*

The genome structure of the *T. turgidum* × *T. monococcum* synthetic hexaploid lines was characterized by genomic *in situ* hybridization (GISH). Total genomic DNA of *Triticum urartu* was labelled with digoxigenin and used as an A-genomic probe, while biotin-labelled DNA from *Aegilops speltoides* was applied as a B-genomic probe for the discrimination of the A and B genomes. Digoxigenin and biotin were detected using anti-digoxigenin-rhodamine and streptavidin-FITC, respectively. The GISH procedure was carried out according to Molnár et al. (2009).

Results

The first step in producing *T. turgidum* × *T. monococcum* synthetic amphiploids is the identification of einkorn accessions with good crossability. A crossing programme was carried out to investigate the crossability of selected einkorn lines and accessions.

Most crosses with traditional einkorn accessions have failed to produce seeds, except the combinations durum × ID140 and emmer × MVGB361, where very low seed set was obtained (Table 1). Better results were achieved in crosses with semi-dwarf einkorn lines, where the highest seed set percentage was >20%.

Table 1
Results of crossability between tetraploid and diploid *Triticum* genotypes

Tetraploid species ♀	Einkorn genotypes ♂	No. of flowers pollinated	No. of seeds obtained	Seed set (%)
	ID140	273	2	0.7
	Mv-Alkor	1247	0	0
	MVGB361	1147	0	0
	MVGB747	570	0	0
Triticum turgidum ssp. *durum*	G11	640	0	0
MVTD14-04	1T-1	398	96	24.1
	2T-1	429	84	19.6
	3T-1	530	87	16.4
	4T-1	568	5	0.9
	3T-3	720	187	25.9
	ID140	671	0	0
	Mv-Alkor	840	0	0
	MVGB361	720	2	0.3
Triticum turgidum ssp. *dicoccon*	MVGB747	572	0	0
Mv Hegyes	G11	946	0	0
	1T-1	235	45	19.1
	2T-1	358	61	17
	3T-1	339	69	20.4
	4T-1	341	1	0.3
	3T-3	421	18	4.3

Significant differences in crossability between the two tetraploid subspecies were observed. The best seed set was obtained for durum × 3T-3 crosses (25.9%), while the emmer × 3T-3 crosses gave the second lowest seed set (4.3%).

The F_1 seeds derived from traditional einkorn accessions did not germinate or perished after germinating. By contrast, the germination rate of F_1 seeds derived from semi-dwarf einkorn lines was high and fertile plants were produced after colchicine treatment (Table 2).

Table 2
Germination and fertility of the *Triticum turgidum* × *T. monococcum* ssp. *monococcum* synthetic amphiploids

Combination	No. of hybrid seeds	Germination (%)	No. of hybrid plants	No. of fertile plants	No. of F_2 seeds
MVTD14-04 × 1T-1	96	100	95	90	236
MVTD14-04 × 2T-1	80	100	79	65	187
MVTD14-04 × 3T-1	87	76	38	32	156
MVTD14-04 × 4T-1	0	0	0	0	0
MVTD14-04 × 3T-3	160	81	29	14	37
Mv Hegyes × 1T-1	21	100	17	17	58
Mv Hegyes × 2T-1	61	100	42	31	49
Mv Hegyes × 3T-1	51	82	29	26	67
Mv Hegyes × 4T-1	0	0	0	0	0
Mv Hegyes × 3T-3	12	50	5	5	24

Genome duplication was performed on seeds proved cytologically to be triploid, and highly fertile amphiploids were developed. Two-colour GISH clearly discriminated the A and B genome chromosomes on the basis of their intense green and orange fluorescent signals, respectively (Fig. 1). Consequently,

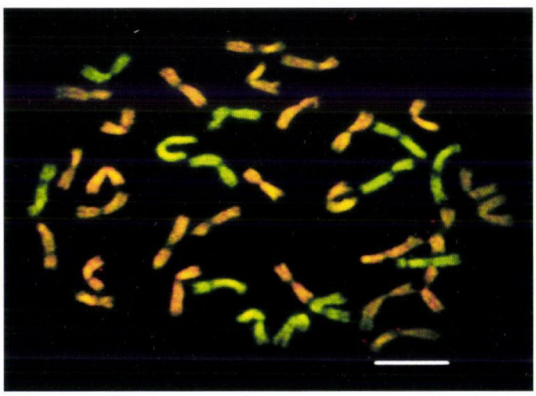

Fig. 1. Two-colour GISH on mitotic chromosomes of synthetic hexaploid wheat (*Triticum turgidum* ssp. *durum* × *T. monococcum*). The chromosomes of the B genome are green, while the A^u and A^m chromosomes are orange. Bar = 10 μm

the A^uBA^m genome structure of the new amphiploids was confirmed. However, the A^u and A^m chromosomes cannot be distinguished with this method.

The F_3 progenies of durum × einkorn crosses and the F_2 progenies of emmer × einkorn crosses were studied for agronomic performance, disease resistance and genetic variability in the nursery (Table 3). Practically all the lines and genotypes developed showed excellent resistance against certain wheat diseases (leaf rust, powdery mildew), but not against fusarium.

Table 3

Observation data on *Triticum turgidum* × *T. monococcum* ssp. *monococcum* synthetic amphiploid lines in the nursery (Martonvásár, 2010)

Genotypes	Heading date	Plant height (cm)	Leaf rust (0–4)	Powdery mildew (0–9)
Durum × einkorn lines	21 May–4 June	60–135	0	0
Emmer × einkorn lines	1–3 June	150–160	0	0
Durum parent MVTD14-04	21 May	70	3	3
Emmer parent Mv Hegyes	3 June	160	0	0
Einkorn parent 1T-1	25 May	75	0	0
Bread wheat control Mv Marsall	20 May	60	3	4

Comparing the two amphiploid combinations, lines originating from the durum × einkorn cross had better straw strength and earlier heading than emmer-based lines. Moreover, durum-based amphiploid lines exhibited a very high level of genetic diversity in terms of plant height, flowering time, ear structure and form, and plant growth habit. By contrast, no visible phenotypic variability was observed among the emmer-based amphiploids.

One unfavourable attribute of the newly developed amphiploids was that many durum × einkorn lines had brittle rachis. These lines were rejected during selection. This feature was not typical of amphiploids derived from emmer × einkorn crosses.

Discussion

Crossability is the most important factor for developing amphiploids. The crossability of several wild and cultivated einkorn accessions with tetraploid and hexaploid *Triticum* species was studied by The and Baker (1975), who found that the cultivated einkorn accessions were less crossable than the wild einkorns and that tetraploid *Triticum* species could be crossed with einkorn more easily than the

hexaploids. These findings suggest that developing synthetic amphiploids using tetraploid *Triticum* and einkorn is a faster and more effective way of utilizing einkorn in wheat breeding than direct crosses between einkorn and hexaploid wheat. The crossability of different *Triticum* species was also studied by Bhagyalakshmi et al. (2008), who obtained no seed set in einkorn × durum or einkorn × emmer crosses.

In the present study on the crossability of different cultivated einkorn accessions a substantial difference was found between the traditional type of cultivated accessions and the semi-dwarf einkorn breeding lines. The semi-dwarf einkorn lines were produced using mutagenesis (G. Kovács, personal communication) from a cultivated einkorn accession. Mutagenesis may generate chromosomal rearrangements, which are probably responsible for the significant difference in crossability. The two einkorn lines with the best crossability (1T-1 and 3T-3) were selected for further crosses.

Differences were also observed in the heading and flowering dates of the parental species. On the basis of several years' observation in the nursery, the semi-dwarf einkorn lines usually have earlier heading and flowering than the traditional types, but the difference is only 2–3 days.

The main goal of developing interspecific hybrids is the transfer of useful genes into cultivated plants. Several studies reported on the possible use of amphiploids with A^uBA^m genome structure in wheat breeding. Durum × einkorn synthetic amphiploids have been used to transfer resistance to Karnal bunt from *T. monococcum* ssp. *aegilopoides* to hexaploid wheat (Kuraparthy et al., 2000).

Several *T. monococcum* × *T. turgidum* ssp. *durum* amphiploids (genome AAAABB) were tested for stripe rust resistance (Ma et al., 1997), for Karnal bunt (Multani et al., 1988) and for powdery mildew and leaf rust resistance (Plamenov et al., 2009) to determine the most resistant lines, which could be useful genetic resources for wheat breeding.

Quality is also a major objective in wheat breeding. Lage et al. (2006) examined the quality of emmer-based synthetic hexaploid wheat lines, and suggested that the genetic variation for quality in tetraploid emmer wheat could be transferred to synthetic hexaploid wheat and combined with plump grains and high grain weight, to be used for bread wheat breeding. However, a further evaluation of the newly synthesized amphiploids is needed in point of quality parameters and antioxidant content.

In conclusion, the newly produced *T. turgidum* × *T. monococcum* synthetic hexaploids are promising genetic resources for wheat breeding. Further investigations have been started to combine the AG genome of *Triticum timopheevii* Zhuk. and the AD genome of *Triticum erebuni* Gandil. with the A^m genome of einkorn genotypes with good crossability.

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References

Barnabás, B., Pfahler, P. L., Kovács, G. (1991): Direct effect of colchicine on the microspore embryogenesis to produce dihaploid plants in wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.,* **81**, 675–678.

Bhagyalakshmi, K., Vinod, K. K., Kumar, M., Arumugachamy, S., Prabhakaran, A. J., Raveendran, T. S. (2008): Interspecific hybrids from wild × cultivated *Triticum* crosses – A study on the cytological behaviour and molecular relations. *J. Crop Sci. Biotech.,* **11**, 257–262.

Brandolini, A., Hidalgo, A., Moscaritolo, S. (2008): Chemical composition and pasting properties of einkorn (*Triticum monococcum* L. subsp. *monococcum*) whole meal flour. *J. Cer. Sci.,* **47**, 599–609.

Gill, R. S., Dhaliwal, H. S., Multani, D. S. (1988): Synthesis and evaluation of *Triticum durum – T. monococcum* amphiploids. *Theor. Appl. Genet.*, **75**, 912–916.

Goncharov, N. P., Bannikova, S. V., Kawahara, T. (2007): Wheat artificial amphiploids involving the *Triticum timopheevii* genome: their studies, preservation and reproduction. *Genet. Resour. Crop Evol.*, **54**, 1507–1516.

Kuraparthy, V., Harjit, S., Parveen, C., Singh, S., Dhaliwal, H. S. (2000): Molecular tagging of Karnal bunt resistance genes of *Triticum monococcum* L. transferred to *Triticum aestivum* L. *Crop Improvement,* **27,** 33–42.

Lage, J., Skovmand, B., Peña, R. J., Andersen, S. B. (2006): Grain quality of emmer wheat derived synthetic hexaploid wheats. *Genet. Resour. Crop Evol.*, **53**, 955–962.

Ma, H., Singh, R. P., Mujeeb-Kazi, A. (1997): Resistance to stripe rust in durum wheats, A-genome diploids, and their amphiploids. *Euphytica*, **94**, 279–286.

Molnár, I., Benavente, E., Molnár-Láng, M. (2009): Detection of intergenomic chromosome rearrangements in irradiated *Triticum aestivum – Aegilops biuncialis* amphiploids by multicolour genomic *in situ* hybridization. *Genome,* **52**, 156–165.

Monneveux, P., Zaharieva, M., Rekika, D. (2000): The utilization of *Triticum* and *Aegilops* species for the improvement of durum wheat. pp. 71–81. In: Royo, C., Nachit, M. M., Di Fonzo, N., Araus, J. L. (eds.), *Durum Wheat Improvement in the Mediterranean Region: New Challenges: L'amélioration du blé dur dans la région méditerranéenne: Nouveaux défis*, CIHEAM-IAMZ, Zaragoza (Spain).

Mujeeb-Kazi, A., Hettel, G. P. (eds.) (1995): Utilizing Wild Grass Biodiversity in Wheat Improvement: 15 Years of Wide Cross Research at CIMMYT. *CIMMYT Research Report No. 2*, CIMMYT, Mexico City, 140 pp.

Mujeeb-Kazi, A., Rosas, V., Roldan, S. (1996): Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmal. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s. lat. × *T. tauschii;* 2n = 6x = 42, AABBDD) and its potential utilization for wheat improvement. *Genet. Resour. Crop Evol.,* **43**, 129–134.

Multani, D. S., Dhaliwal, H. S., Singh, P., Gill, K. S. (1988): Synthetic amphiploids of wheat as a source of resistance to Karnal bunt *(Neovossia indica). Plant Breed.,* **101**, 122–125.

Plamenov, D., Belchev, I., Kiryakova, V., Spetsov, P. (2009): Fungal resistance of *Triticum durum* – *T. monococcum* ssp. *aegilopoides* amphiploid. *J. Plant Dis. Protect.,* **116**, 60–62.

The, T. T., Baker, E. P. (1975): Basic studies relating to transference of genetic characters from *Triticum monococcum* L. to hexaploid wheat. *Aust. J. Biol. Sci*., **28,** 189–199.

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EFFECT OF CO_2 ENRICHMENT ON CANOPY PHOTOSYNTHESIS, WATER USE EFFICIENCY AND EARLY DEVELOPMENT OF TOMATO AND PEPPER HYBRIDS

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The effect of CO_2 enrichment on the rate of photosynthesis and the water use efficiency (WUE) of young pepper and tomato plants was studied in the phytotron. A CO_2 level of 1000 ppm significantly increased the net assimilation rate in the upper foliage, while the increase was even more considerable in the lower layers of the canopy, with values of up to 100%. The 1500 ppm CO_2 level caused a further substantial increase in CO_2 assimilation and at least doubled (in tomato) or tripled (in pepper) the water use efficiency on a leaf area basis compared to the ambient values. Although the response in terms of photosynthesis and WUE was not variety-specific, there were differences between the pepper hybrids in the biomass components, exceeding 100% for the total biomass at the 1500 ppm CO_2 level. In tomato, however, there was no significant variation in the total biomass of the three hybrids investigated in this early phase of development at either CO_2 level.

Key words: elevated CO_2, pepper, tomato, photosynthesis, WUE

Introduction

As CO_2 is a limiting factor for photosynthesis in C_3 plants, a rise in the atmospheric CO_2 level can be expected to increase the rate of assimilation and enhance dry matter production (Kramer, 1981; Lawlor and Mitchell, 1991). The rate of stimulation is, however, greatly influenced by the growth type and the nutrient supplies (Poorter et al., 1996; Li et al., 2007). The positive effect of CO_2 enrichment is well known and has been applied in horticulture for various species for a long time (Tremblay and Gosselin, 1998; Terbe and Slezák, 2008). CO_2 fertilization can improve fruit yield and composition, and can cause earlier ripening (Wheeler et al., 1997; Csuvár et al., 2009). The early vegetative growth of young plants may be stimulated even more, resulting in heavier transplants, desirable for successful field establishment, without elongation growth (Woodrow et al.,

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1987). In tomato transplants, the shoot and root mass and leaf dry weight were demonstrated to increase by 81%, while transpiration decreased by 34% (Woodrow et al., 1987). In a study involving 96 tomato genotypes, young plant growth increased 2.3-fold at the doubled CO_2 level (Lindhaut and Pet, 1990). CO_2 levels higher than 600–900 ppm, however, are likely to affect plants less favourably (Mortensen, 1987).

As atmospheric CO_2 enrichment can be applied successfully in horticulture, the CO_2 emitted by industry could be a possible source for use in plant production. Besides the economic benefits this would also lead to a reduction in total CO_2 emissions and less burdening of the environment. One example in Hungary could be the use of flue gases, originating from the burning of natural gas, for hydrogen production at the Danube Refinery (MOL Group) in Százhalombatta (MOL DSD, 2010; MOL, 2011).

As most research on photosynthesis was associated with the light-saturated rate of assimilation, one aim of the present work was to examine how much the actual photosynthetic intensity is stimulated by CO_2 enrichment and to compare responses in different canopy layers. CO_2 concentrations of 1000 and 1500 ppm were examined to reveal differences in the reactions of various pepper and tomato hybrids in terms of photosynthesis, water use efficiency and biomass components. The relationships between photosynthetic parameters and the actual accumulation of biomass components were also investigated in different hybrids at various CO_2 levels to find out if they have a predictive value for the biomass accumulation potential.

Materials and methods

Preliminary experiment

Six-week-old plants of sweet pepper (*Capsicum annuum* L.) hybrid Kurca RZ F1, and tomato (*Lycopersicon esculentum* Mill.) hybrid Petula F1 were planted in 3 L pots containing the growth medium E-950 SPEZIAL SUBSTRAT (Stender AG Schermbeck, Germany) and placed in two PGB-96 growth chambers. The distances between the plants were 20 cm for pepper and 35 cm for tomato, while there were approximately 60 cm between the two rows of pots for both the pepper and tomato growth chambers. The plants were irrigated regularly and received nutrients as foliar fertilization (Volldünger Linz) in the 5th week after planting. The growth conditions were a 16/8 h day-night regime with a temperature of 24/20°C, while the light intensity was 300 μmol m^{-2} s^{-1}. The CO_2 level was maintained at either 400 or 1000 ppm (during light hours).

Photosynthesis measurements were carried out on four individual plants six weeks after planting using a LiCOR-6400 portable photosynthesis system (Lincoln, Nebraska, USA). Net assimilation rates were recorded at two canopy levels (lower and upper) in pepper and three levels in tomato (low, middle and top) in each CO_2 treatment at a PAR value of 250 m^{-2} s^{-1}.

Measurements on the variation in pepper and tomato hybrids

Six-week-old plants of sweet pepper hybrids (Kurca RZ F1 and Hó F1), hot chilli pepper (Darázs F1) and tomato hybrids (Petula F1, Tourance F1 and Flexxion F1) were planted in 10 L pots

containing the growth medium E-950 SPEZIAL SUBSTRAT (Stender AG Schermbeck, Germany). The plants were placed randomly in three PGB-96 growth chambers in which the atmospheric CO_2 levels were 400 (ambient), 1000 and 1500 ppm. The distances between plants and rows were 35 and 60 cm. The temperature was 23/19°C during the 15/9 h day-night cycle, while the light intensity during growth was 200–230 μmol m^{-2} s^{-1}. The plants were irrigated regularly and received 3 dL of 10 g/L Volldünger Linz nutrient solution per pot once a week in the control, and 1.5 times and twice in the case of pepper, and twice and three times a week in the case of tomato at 1000 and 1500 ppm, respectively, starting from the third week after planting. Net assimilation rate (Pn) and transpiration (Tr) were measured at a PAR value of 230 μmol photons m^{-2} s^{-1} with a CIRAS-2 portable photosynthesis meter (PP Systems, Amesbury, USA). The water use efficiency (WUE) was calculated as the ratio of Pn (μmol CO_2 m^{-2} s^{-1}) to Tr (mmol H_2O). Six weeks after planting, two plants from each variety and treatment were analysed for leaf area (LI-3000C Portable Area Meter) and biomass components. Dry weight was determined after 24 h desiccation at 100°C.

Two-way ANOVA (BREEDER software, Láng et al., 2001) was used to compare the effect of CO_2 level and the genotype, while in the preliminary experiment the two factors were the CO_2 level and measurement height for each variety. Correlation analysis was applied on the data set of different CO_2 levels and genotypes to study the relationships between different parameters (Microsoft Excel 2007).

Results

Effect of CO_2 enrichment on photosynthesis at different canopy levels

Substantial increases in the net assimilation rate were recorded for both species in response to the high CO_2 level (Fig. 1). Though the rise in photosynthesis was significant even at the top of the plants, this increase was much more pro-

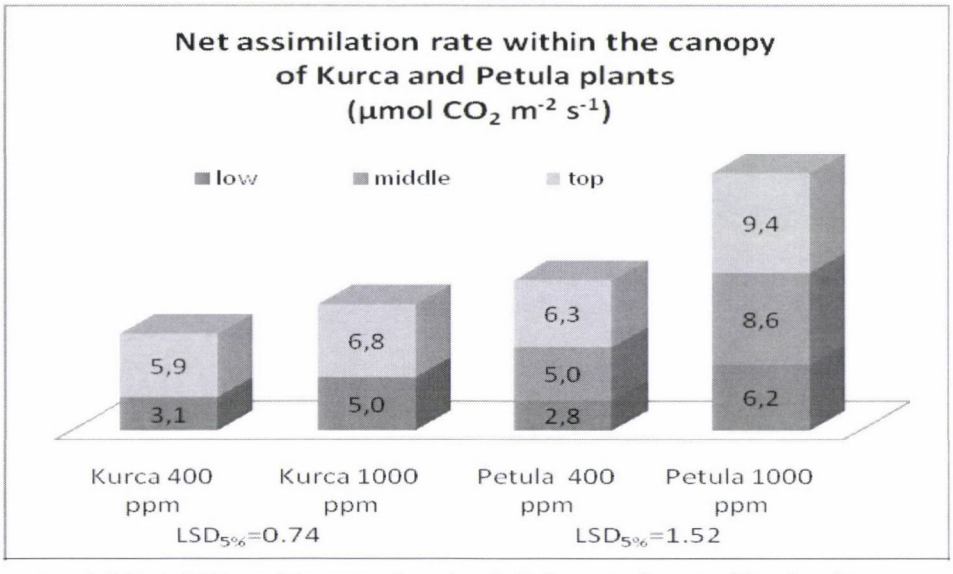

Fig. 1. Effect of CO_2 enrichment on the net assimilation rate of pepper (Kurca) and tomato (Petula) plants 6 weeks after planting at the 400 and 1000 ppm CO_2 levels

nounced (up to more than 100%) in the lower layers of the foliage, where the light intensity was poorer. In plants grown at elevated CO_2 (1000 ppm), the rate of photosynthesis in the lowest leaves was nearly as high as in the light-leaves of the control plants.

Change in net photosynthesis and water use efficiency in a variety of pepper and tomato hybrids, 6 weeks after planting

An additional rise in the CO_2 level resulted in a further increase in photosynthetic activity (Fig. 2). The growth was higher in pepper, where photo-

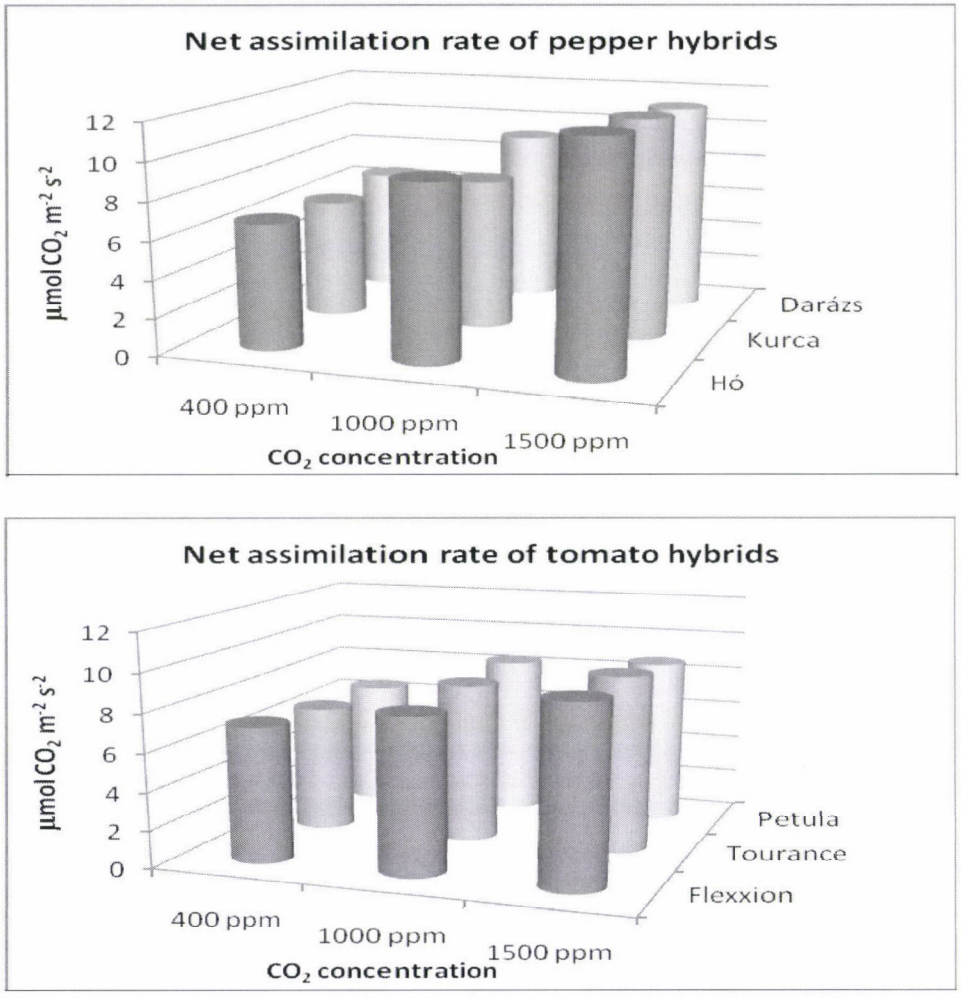

Fig. 2. Effect of CO_2 enrichment on the net assimilation rate in pepper and tomato hybrids

synthesis was 26–40% more intense at 1000 ppm and 71–85% higher at the 1500 ppm CO_2 level. The corresponding increase in tomato was 15–29% and 32–40%, respectively. There were only slight, non-significant differences in the absolute values of photosynthesis between the different hybrids of each species.

CO_2 enrichment resulted in very great increases in the water use efficiency, which was 76–114% higher in pepper and 80–117% higher in tomato at the 1000 ppm CO_2 level. At 1500 ppm it was about three times the control value in pepper and twice or slightly less than three times the control value in tomato (Fig. 3). However, the gain in assimilation compared to the water loss during transpiration was definitely more economical in pepper than in tomato at the highest CO_2 level. Differences between the various hybrids of each species were not distinct in either CO_2 treatment.

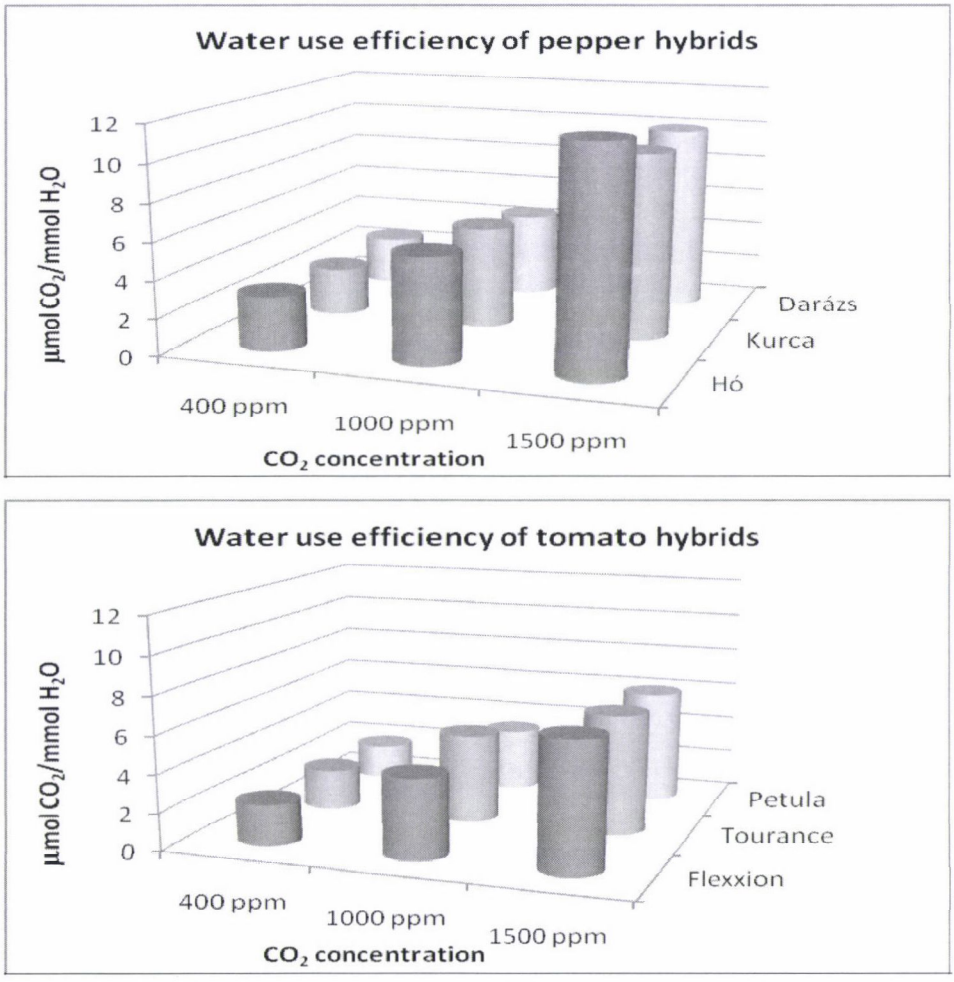

Fig. 3. Water use efficiency at various CO_2 levels 6 weeks after planting

The biomass accumulation till the 6th week after planting, averaged over the varieties, was greatly enhanced by elevated CO_2, though the extent of change was not significant in all the hybrids (Fig. 4). In pepper, CO_2 enrichment significantly increased the mass of root, stem and leaf, and the leaf area. Darázs had the largest root and stem weights, while Hó had the most leaf area and mass (though not significantly different from Darázs). Kurca had the lowest values for most biomass components. The difference between the hybrids was most extreme at the 1500 ppm CO_2 level, where the total dry biomass of Darázs was double that of Kurca.

The extent of increase due to high CO_2 was less pronounced for tomato hybrids and did not reveal significant differences between the hybrids in terms of to-

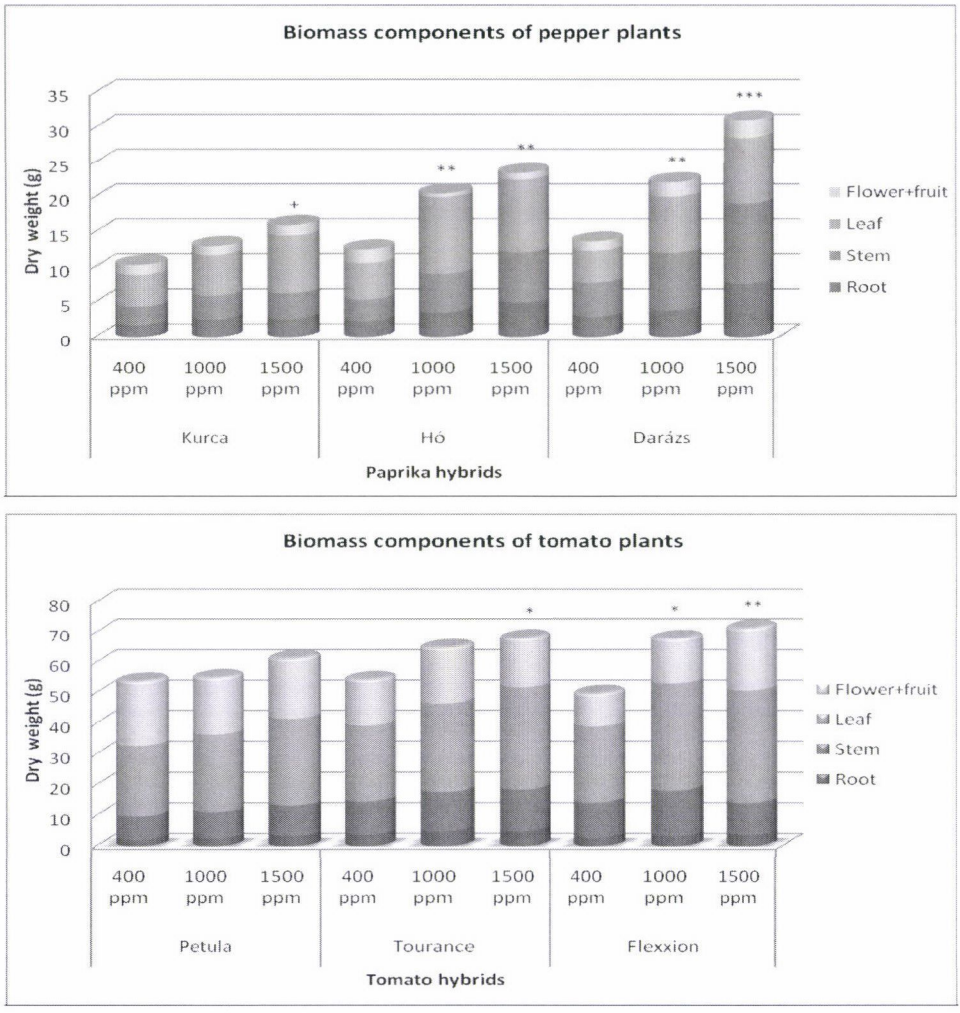

Fig. 4. Effect of CO_2 enrichment on the biomass accumulation of pepper and tomato 6 weeks after planting

tal biomass accumulation. Only the highest CO_2 level resulted in significant rises in the root mass and leaf area of tomato plants, while there was no difference in the stem weights. Leaf mass only increased significantly in Tourance and Flexxion at 1500 ppm CO_2, compared to the ambient values. Though the relative increase in leaf area was less than in pepper, in absolute terms it was considerable, rising by as much as 0.6–1.4 m^2 per plant, resulting in a total leaf area of 5.6–6.0 m^2. The leaf area increase in response to the highest CO_2 level was greater in pepper (50–60%), but the total leaf area was much smaller than in tomato, ranging from 2.1–2.4 m^2 per plant.

Relationship between photosynthetic parameters and biomass components

The rate of net photosynthesis showed a very close relationship with the total biomass accumulation in both species. The leaf weight and area were also in high positive correlation with Pn, while no significant relationship was found between the root and stem weights and the photosynthetic activity, suggesting that these parameters might be influenced by other factors, such as the genotype effect.

A negative correlation was found between transpiration and the biomass components, underlining the phenomenon that plants tend to lose less water at elevated CO_2 levels yet produce higher biomass yields. Besides the leaf and total biomass parameters, the root fresh weight in pepper and the stem fresh weight in tomato exhibited positive correlations with the water use efficiency.

Discussion

It was suggested previously that it is only worth applying higher CO_2 levels if the light intensity is optimum (Fierro et al., 1993), but it was demonstrated here that in both pepper and tomato photosynthesis is greatly enhanced by high CO_2 level even at low light intensity. The assimilation rate of the lower shade-leaves at high CO_2 was very similar to the values recorded for the upper light-leaves at ambient level.

Despite the fact that some authors do not recommend the application of CO_2 concentrations of more than 1000 ppm (Mortensen, 1987), the highest CO_2 concentration investigated (1500 ppm) was found to have the most favourable effect on both species. Photosynthesis was greatly stimulated, water use efficiency improved, and the leaf area and dry matter production increased considerably. There was, however, considerable variation among the pepper hybrids, while the tomato hybrids, which were probably greatly improved varieties, did not show any significant differences in their response to high CO_2.

On average, the rise in the root, stem and leaf mass and the total biomass in response to 1000 ppm CO_2 was 44%, 57%, 73% and 51% for pepper and 22%, 20%, 22% and 19% for tomato hybrids, respectively. This is higher than the re-

Table 1
Correlation coefficients of the correlation analysis between the photosynthetic and morphological parameters of three hybrids each of pepper and tomato grown at CO_2 levels of 400, 1000 and 1500 pm

Pepper	Pn	Tr	WUE	Tomato	Pn	Tr	WUE
Root FW	0.6642	–0.6413	**0.6700**	Root FW	0.5283	–0.6625	0.6029
Stem FW	0.6328	–0.5430	0.5514	Stem FW	0.6486	**–0.6740**	**0.7062**
Leaf FW	**0.7433**	**–0.7511**	**0.6740**	Leaf FW	**0.8041**	**–0.7967**	**0.8608**
Leaf area	**0.8027**	**–0.7799**	**0.7088**	Leaf area	**0.6771**	–0.6065	0.6506
AGB FW	**0.7554**	**–0.6893**	**0.6616**	AGB FW	**0.8541**	**–0.8793**	**0.9107**
Total biomass FW	**0.7428**	**–0.6899**	**0.6809**	Total biomass FW	**0.8603**	**–0.8988**	**0.9217**
Root DW	0.6431	–0.6211	0.6432	Root DW	0.5037	–0.6500	0.5889
Stem DW	0.5827	–0.4873	0.5184	Stem DW	0.1664	–0.3043	0.2094
Leaf DW	**0.8469**	**–0.7985**	**0.7590**	Leaf DW	**0.6962**	**–0.7328**	**0.7550**
AGB DW	**0.7640**	**–0.6768**	**0.6796**	AGB DW	**0.8026**	**–0.8755**	**0.8580**
Total biomass DW	**0.7432**	**–0.6721**	**0.6802**	Total biomass DW	**0.8068**	**–0.8929**	**0.8689**

Numbers in bold represent significant relationships at the $p \leq 0.05$ probability level.
Pn = net photosynthesis (net assimilation rate), Tr = transpiration, WUE = water use efficiency, FW = fresh weight, DW = dry weight, AGB = above-ground biomass

ported increases of 4–15% in the roots, 3–10% in the stems, 6–12% in the leaves and 6–10% in the total biomass of tomato plants at various nutrient levels, when exposed to doubled atmospheric CO_2 (Li et al., 2007). In the present study, the 1500 ppm CO_2 concentration stimulated the total biomass accumulation even more; the rise in the total biomass was 90% for pepper and 27% for tomato. The results for tomato were similar to those found by other authors, where CO_2 concentrations of 1050 and 1400 ppm stimulated the dry biomass of tomato plants by 17 and 23%, 66 days after sowing (van Oosten et al., 1995).

At 1500 ppm WUE was shown here to increase 2–3-fold in tomato and 3-fold in pepper, which was similar to what was found for tomato plants at twice the ambient CO_2 level (Maggio et al., 2002), for unstressed plants (doubled value) and under severe salt stress (more than three times the value). As the leaf area was also found to increase greatly in the present work, the total canopy water use can be assumed to be higher than that recorded on a leaf area basis (Grodzinski et al., 1986).

Enhanced growth and dry matter accumulation were found to be correlated with higher net photosynthetic rates in young vegetative tissues in the case of CO_2 enrichment (Tremblay and Gosselin, 1998). Under the present conditions, not only photosynthesis but also WUE proved to be related to biomass accumulation (negative correlation). The strongest relationships for the net assimilation rate and WUE were found with the total biomass and the leaf parameters.

The present results confirmed that very high CO_2 levels can indeed have a large positive effect on the development of both tomato and pepper. Although there was considerable variation among the pepper hybrids, the tomato varieties

were more similar in their reaction to high CO_2. Photosynthesis was greatly stimulated, resulting in a considerable rise in the organic matter production and also in higher leaf area. The water use efficiency on a leaf area basis was greatly improved at 1500 ppm CO_2 concentration, leading to a substantial decrease in the water consumption of the plants.

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References

Csuvár, Á., Krecz, Á., Paksi, A., Kassai, T., Dimény, J. (2009): Effect of atmospheric CO_2 enrichment on tomato grown in soil. *Cereal Res. Commun.,* **37**, 201–204.

Fierro, A., Tremblay, N., Gosselin, A. (1993): CO_2 enrichment and supplementary lighting improve growth and yield of tomato and pepper transplants. *HortScience,* **29**, 152–154.

Grodzinski, B., Woodrow, L., Leonardos, E. D., Dixon, M., Tsujita, M. J. (1986): Plant responses to short- and long-term exposures to high carbon dioxide levels in closed environments. *Adv. Space Res.,* **18**, 203–211.

Kramer, P. J. (1981): Carbon dioxide concentration, photosynthesis, and dry matter production. *Bioscience,* **31**, 29–33.

Láng, L., Kuti, C., Bedő, Z. (2001): Computerized data management system for cereal breeding. *Euphytica,* **119**, 235–240.

Lawlor, D. W., Mitchell, R. A. C. (1991): The effects of increasing CO_2 on crops photosynthesis and productivity: A review of field studies. *Plant Cell Environ.,* **14**, 807–818.

Li, J., Zhou, J-M., Duan, Z-Q., Du, C-W., Wang, H-Y. (2007): Effect of CO_2 enrichment on the growth and nutrient uptake of tomato seedlings. *Pedosphere,* **17**, 343–351.

Lindhaut, P., Pet, G. (1990): Effects of CO_2 enrichment on young plant growth of 96 genotypes of tomato (*Lycopersicon esculentum*). *Euphytica,* **51**, 191–196.

Maggio, A., Dalton, F. N., Piccini, G. (2002): The effects of elevated CO_2 on static and dynamic indices for tomato salt tolerance. *Eur. J. Agron.,* **16**, 197–206.

MOL DSD (2010): Vizsgálati Jegyzőkönyv A Dunai Finomító Hidrogéngyár-2 reformáló kemencéjének emissziós vizsgálatairól. (Determination of emission of the reforming furnace in Hydrogen unit No. 2 of Danube Refinery – Test Report). 11 pp.

MOL (2011): MOL Group Annual Report 2010 – Economic, social and environmental performance. MOL Hungarian Oil and Gas Plc, http://www.mol.hu/annualreport2010/uploads/file/2010/pdf/en1.pdf; 258 pp.

Mortensen, L. M. (1987): Review: CO_2 enrichment in greenhouses. Crop responses. *Scientia Hort.,* **33**, 1–25.

Poorter, H., Roumet, C., Campbell, B. D. (1996): Interspecific variation in the growth response of plants to elevated CO_2: A search for functional types. pp. 375–412. In: Körner, C., Bazzaz, F. A. (eds.), *Carbon Dioxide. Populations, and Communities.* Academic Press, New York.

Terbe, I., Slezák, K. (eds.) (2008): *Talaj nélküli zöldséghajtatás.* (Vegetable production in soilless cultures.) Mezőgazda Press, Budapest. 372 pp.

Tremblay, N., Gosselin, A. (1998): Effect of carbon dioxide enrichment and light. *HortTechnology October,* **8**(4), 5.

van Oosten, J. J., Wilkins, D., Besford, R. T. (1995): Acclimation of tomato to different carbon dioxide concentrations. Relationship between biochemistry and gas exchange during leaf development. *New Phytol.,* **130**, 357–367.

Wheeler, R. M., Mackowiak, C. L., Stutte, G. W., Yorio, N. C., Berry, W. L. (1997): Effect of elevated carbon dioxide on nutritional quality of tomato. *Adv. Space Res.,* **20**, 1975–1978.

Woodrow, L., Liptay, A., Grodzinski, B. (1987): The effects of CO_2 enrichment and etephon application on the production of tomato transplants. *Acta Hort.,* **201**, 133–140.

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MITOGEN-ACTIVATED PROTEIN (MAP) KINASE SIGNALLING IN PLANT ENVIRONMENTAL STRESS RESPONSES

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Due to their sessile life style plants have to cope with a variety of unfavourable environmental conditions. Extracellular stimuli are perceived by specific sensors and receptors and are transmitted within the cell by various signal transduction pathways to trigger appropriate responses. The mitogen-activated protein (MAP) kinase cascades are well-conserved signalling pathway modules found in all eukaryotes. Activated MAP kinases phosphorylate an array of substrate proteins. Phosphorylation results in altered substrate activities that mediate a wide range of responses, including changes in gene expression. The genome of the model plant *Arabidopsis thaliana* contains genes encoding 20 mitogen-activated protein kinases and 10 MAPK kinases. In plants MAP kinases play a central role in environmental stress signalling; however, our knowledge mainly comes from results on three MAP kinases and their immediate upstream activators. Further studies on additional members of the plant MAP kinase repertoire together with the identification of downstream substrates and connections to specific upstream signal receptors are required to elucidate their specific functions within environmental stress signalling networks. Understanding the mechanisms of specificity in signal flow is indispensable for engineering improved crops with modified MAP kinase signalling for agricultural purposes.

Key words: environmental stress, signal transduction, MAP kinase, *Arabidopsis thaliana*

Introduction: The canonical MAP kinase cascade

The mitogen-activated protein (MAP) kinases, discovered approximately 20 years ago, together with their immediate upstream regulators, are among the most studied signal transduction molecules (Avruch, 2007). They function as the most downstream members of hierarchical phosphorylation cascades known as MAP kinase cascades. In a canonical MAP kinase cascade, signals are transmitted

by sequential phosphorylation events: kinases at each level are activated by phosphorylation and in turn phosphorylate (and thus activate) their downstream counterparts. A canonical MAP kinase cascade consists of three types of enzymes: the MAP kinase (MAPK or MPK), a MAP kinase kinase (MKK), and a MAP kinase kinase kinase (MAPKKK or MAP3K). The activation of a MAP kinase cascade often occurs within one to several minutes of stimulation, representing one of the earliest cellular responses to environmental cues. MAPK phosphorylation cascades are conserved signalling modules in all eukaryotes and known to have pivotal roles in regulating ubiquitous processes such as cell division, growth and stress responses. In plants MAP kinase pathways have been shown to play a major role in stress signalling and their roles in various aspects of plant development have also been explored (Colcombet and Hirt, 2008).

This review highlights the central role of MAP kinase signalling pathways in environmental stress signalling in the model plant *Arabidopsis thaliana*, and present limitations in our knowledge regarding signal specificity. Despite their obvious biological importance, the number of MAP kinase components is limited. Yeast has six MAPKs and mammals 14, while the *A. thaliana* genome encodes 20–23 MAPKs. The mammalian MAPKs can be divided into four distinctly regulated groups; the extracellular-signal-related kinases (ERK)–1/2, the Jun amino-terminal kinases (JNK) 1/2/3, the p38 proteins (p38 $\alpha, \beta, \gamma, \delta$) and ERK5. The ERK group is primarily responsible for transducing mitogenic signals, whereas the p38 and JNK groups mostly relay stress signals (Chang and Karin, 2001). Plant MAPKs are not divided into the same functional groups as those of yeast and animals, and all plant MAPKs reported to date belong to the ERK subfamily, although three *Arabidopsis* MAPKs (MPK21-23) have sequence features related both to MAPKs and to cyclin-dependent kinases (CDKs) (Jonak et al., 2002; MAPK Group, 2002).

MKKs lie upstream of the MAPKs. They are the least diverse members of the plant cascades and were therefore suggested to act as points of intersection and integration between converging signals from upstream MAPKKKs and divergent outputs to downstream MAPKs. Sequence analysis has placed the plant MKKs into four groups (A–D) (Jonak et al., 2002; MAPK Group, 2002).

The MAPKKKs form the largest family of MAP kinase components, the *Arabidopsis* genome encoding 60–80 putative members (Champion et al., 2004), but very few members of this family have any assigned biological function. They contain different potential regulatory domains outside the catalytic domain, which means they can be regulated by a variety of upstream signals and then selectively activate MKKs.

MAP kinases are involved in both biotic and abiotic stress signalling

Plants adapt to environmental stresses by changing gene expression patterns, leading to appropriate defence responses. Environmental stress can be ei-

ther abiotic (e.g. drought, soil salinity, cold, heat, wind, ultraviolet (UV) radiation) or biotic (pathogens and pests). MAP kinase signalling cascades have been implicated in plant responses to many types of stresses. An overview of the major stress-signalling MAP kinase pathways is presented in Figure 1.

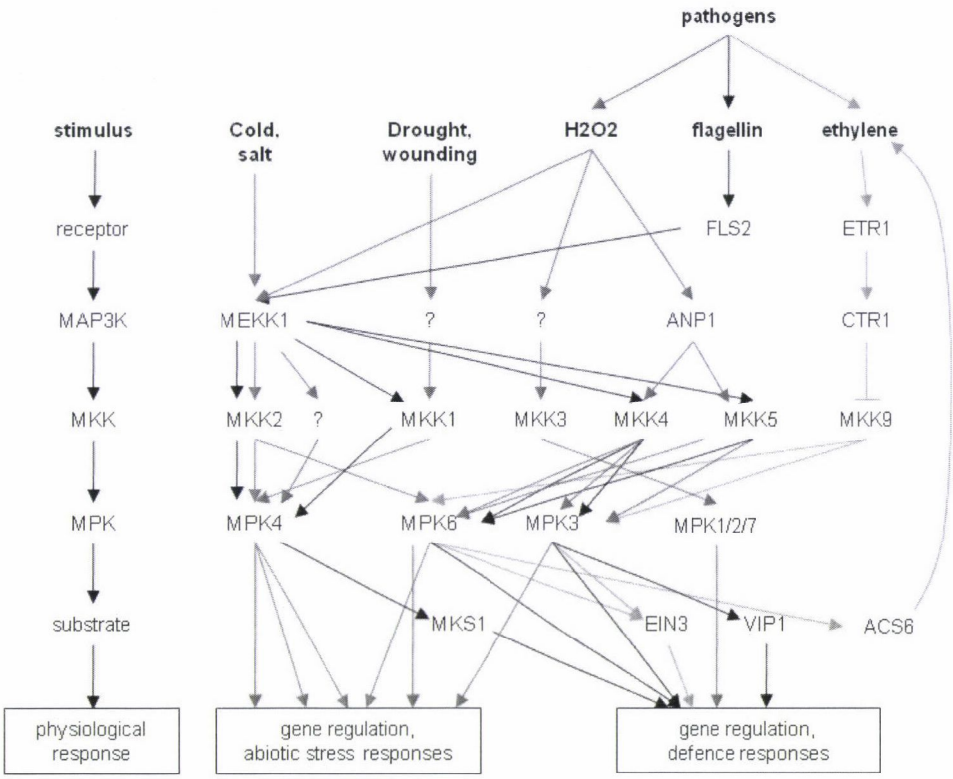

Fig. 1. Overview of the major stress-signalling MAP kinase pathways in *Arabidopsis*. The canonical MAP kinase pathway is shown on the left. Arrows indicate signal flow-path, question marks indicate unknown factors

In *Arabidopsis,* cold, low humidity, hyper-osmolarity, salt stress and wounding rapidly activate MPK4 and MPK6 (Ichimura et al., 2000). Further genetic and biochemical studies revealed a cold- and salt-stress signalling cascade consisting of MEKK1 → MKK2 → MPK4/6 (Teige et al., 2004), while MKK2 and MPK4/6 are also involved in disease resistance (Brader et al., 2007). MEKK1 has also been implicated in the activation of MKK1 in response to wounding signals (Hadiarto et al., 2006). It has also been suggested that MEKK1 may constitute a MAP kinase signalling cascade with MKK4 and MKK5 as well as MPK3 and MPK6 in response to flagellin, a pathogen elicitor (Asai et al., 2002). Accordingly, *MPK6*-silenced *Arabidopsis* plants are compromised in resistance to various pathogens (Menke et al., 2004). MPK4 is also activated by flagellin and recent

genetic evidence suggests that MEKK1 is upstream of MKK1 and MPK4 in flagellin and reactive oxygen species (ROS) signalling (Meszaros et al., 2006; Su et al., 2007; Suarez-Rodriguez et al., 2007). MPK4 is also required for jasmonic acid-responsive gene expression (Petersen et al., 2000).

In a remarkable example of host-pathogen co-evolution, *Agrobacterium* hijacks the MPK3-regulated nucleocytoplasmic shuttle system of VIP1 to transfer its own T-DNA into the plant nucleus where it can integrate into plant genomic DNA (Djamei et al., 2007).

The plant hormone ethylene is produced in response to various stresses, including pathogen attack. Accumulating evidence supports the association of MAP kinase cascades with ethylene. However, there is an ongoing debate regarding whether their actual role is ethylene signal transduction or the regulation of ethylene biosynthesis in response to stresses (Hahn and Harter, 2009).

Emerging data on phylogenetically more distant plant genes show further involvement in stress signalling: for example, MKK3 of group B and MKK7 of group D MAPK kinases participate in pathogen responses (Doczi et al., 2007; Zhang et al., 2007).

The problem of specificity: A few well-known kinases are involved in many stresses

Although our understanding of plant MAP kinases has increased significantly in the past decade, most of this knowledge comes from studies on three MAPKs: MPK3, MPK6 and MPK4. These three kinases are involved in almost all stress signalling and in some developmental processes as well. It is unresolved how signal specificity can be maintained if the same components are involved in so many different processes. The fact that these MAP kinases are activated by reactive oxygen species (ROS), which are common secondary messengers of all stress input signals, may at least in part explain the phenomenon. Alternatively, a MAP kinase could theoretically be a common mediator of several signals if other members and targets of the pathway are expressed in specific cell types, at particular developmental stages or under certain environmental conditions only. Furthermore, it is well known from animal and yeast systems that scaffold proteins and specific docking sites facilitate the recruitment of specific activators and substrates, thereby facilitating specificity.

At present it is difficult to conclude whether being involved in many signalling pathways is a common feature of all the 20 MAPKs or if it is specific for the three well-characterised MAPKs. Therefore, further studies are required to discover the biological functions of the majority of the plant MAPK repertoire. While individual gene functions are best addressed by functional genetics approaches (i.e. studies on mutant lines), intermolecular interactions that constitute the network of phosphorylation cascades can be mapped by biochemical methods. In order to identify potential MKK-MPK modules a systematic pair-wise yeast

two-hybrid analysis was carried out to find interacting MPK-MKK partners (Dóczi unpublished results). This work revealed a high degree of connectivity between group A MKKs and group A and B MPKs, and between group C MKKs and group A MPKs.

In comparison with animal systems very few plant MAP kinase substrates have been described to date, and basically nothing is known about how MAP kinases are connected to upstream stress sensory mechanisms. Future work aiming to characterise the network context of MAP kinase cascades will undoubtedly shed more light on the problem of signal specificity and cross-talk. This knowledge is indispensable for engineering improved crop plants with modified MAP kinase signalling mechanisms for agricultural utilisation.

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References

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W. L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., Sheen, J. (2002): MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature,* **415**, 977–983.

Avruch, J. (2007): MAP kinase pathways: The first twenty years. *Biochim. Biophys. Acta,* **1773**, 1150–1160.

Brader, G., Djamei, A., Teige, M., Palva, E. T., Hirt, H. (2007): The MAP kinase kinase MKK2 affects disease resistance in Arabidopsis. *Mol. Plant Microbe Interact.,* **20**, 589–596.

Champion, A., Picaud, A., Henry, Y. (2004): Reassessing the MAP3K and MAP4K relationships. *Trends Plant Sci.,* **9**, 123–129.

Chang, L., Karin, M. (2001): Mammalian MAP kinase signalling cascades. *Nature,* **410**, 37–40.

Colcombet, J., Hirt, H. (2008): Arabidopsis MAPKs: A complex signalling network involved in multiple biological processes. *Biochem. J.,* **413**, 217–226.

Djamei, A., Pitzschke, A., Nakagami, H., Rajh, I., Hirt, H. (2007): Trojan horse strategy in *Agrobacterium* transformation: Abusing MAPK defense signaling. *Science,* **318**, 453–456.

Doczi, R., Brader, G., Pettko-Szandtner, A., Rajh, I., Djamei, A., Pitzschke, A., Teige, M., Hirt, H. (2007): The *Arabidopsis* mitogen-activated protein kinase kinase MKK3 is upstream of group C mitogen-activated protein kinases and participates in pathogen signaling. *Plant Cell,* **19**, 3266–3279.

Hadiarto, T., Nanmori, T., Matsuoka, D., Iwasaki, T., Sato, K., Fukami, Y., Azuma, T., Yasuda, T. (2006): Activation of *Arabidopsis* MAPK kinase kinase (AtMEKK1) and induction of AtMEKK1-AtMEK1 pathway by wounding. *Planta,* **223**, 708–713.

Hahn, A., Harter, K. (2009): Mitogen-activated protein kinase cascades and ethylene: signaling, biosynthesis, or both? *Plant Physiol.,* **149**, 1207–1210.

Ichimura, K., Mizoguchi, T., Yoshida, R., Yuasa, T., Shinozaki, K. (2000): Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6. *Plant J.,* **24**, 655–665.

Jonak, C., Okresz, L., Bogre, L., Hirt, H. (2002): Complexity, cross talk and integration of plant MAP kinase signalling. *Curr. Opin. Plant Biol.,* **5**, 415–424.

MAP Kinase Group (2002): Mitogen-activated protein kinase cascades in plants: A new nomenclature. *Trends Plant Sci.,* **7**, 301–308.

Menke, F. L., van Pelt, J. A., Pieterse, C. M., Klessig, D. F. (2004): Silencing of the mitogen-activated protein kinase MPK6 compromises disease resistance in *Arabidopsis*. *Plant Cell,* **16**, 897–907.

Meszaros, T., Helfer, A., Hatzimasoura, E., Magyar, Z., Serazetdinova, L., Rios, G., Bardoczy, V., Teige, M., Koncz, C., Peck, S., Bogre, L. (2006): The *Arabidopsis* MAP kinase kinase MKK1 participates in defence responses to the bacterial elicitor flagellin. *Plant J.,* **48**, 485–498.

Nakagami, H., Soukupova, H., Schikora, A., Zarsky, V., Hirt, H. (2006): A mitogen-activated protein kinase kinase kinase mediates reactive oxygen species homeostasis in *Arabidopsis*. *J. Biol. Chem.,* **281**, 38697–38704.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H. B., Lacy, M., Austin, M. J., Parker, J. E., Sharma, S. B., Klessig, D. F., Martienssen, R., Mattsson, O., Jensen, A. B., Mundy, J. (2000): *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. *Cell,* **103**, 1111–1120.

Su, S. H., Suarez-Rodriguez, M. C., Krysan, P. (2007): Genetic interaction and phenotypic analysis of the *Arabidopsis* MAP kinase pathway mutations *mekk1* and *mpk4* suggests signaling pathway complexity. *FEBS Lett.,* **581**, 3171–3177.

Suarez-Rodriguez, M. C., Adams-Phillips, L., Liu, Y., Wang, H., Su, S. H., Jester, P. J., Zhang, S., Bent, A. F., Krysan, P. J. (2007): MEKK1 is required for flg22-induced MPK4 activation in *Arabidopsis* plants. *Plant Physiol.,* **143**, 661–669.

Teige, M., Scheikl, E., Eulgem, T., Doczi, R., Ichimura, K., Shinozaki, K., Dangl, J. L., Hirt, H. (2004): The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. *Mol. Cell.,* **15**, 141–152.

Zhang, X., Dai, Y., Xiong, Y., DeFraia, C., Li, J., Dong, X., Mou, Z. (2007): Overexpression of *Arabidopsis* MAP kinase kinase 7 leads to activation of plant basal and systemic acquired resistance. *Plant J.,* **52**, 1066–1079.

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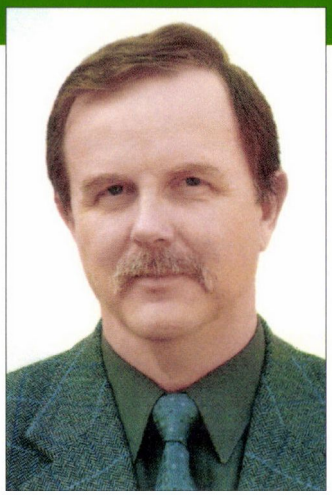

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ELABORATION OF A NON-DESTRUCTIVE METHODOLOGY FOR ESTABLISHING PLANT DEVELOPMENTAL PATTERNS IN CEREALS

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The transitions between various developmental phases are critical in determining the ecological adaptation and yield of cereals. In order to elaborate a methodology for establishing the timing of the consecutive plant developmental phases from germination to the fully developed plant, regular measurements of changes in developmental components were carried out on one winter (Kompolti Korai) and one spring (Morex) barley cultivar in a model experiment. Under the controlled environmental conditions linear regression was characteristic of the associations between the chronological time and all or most of the time course data of plant height, tiller and leaf numbers. The initial growth of the spring barley was twice as intensive as that of the winter barley. The length of the stem elongation phases was similar for the two varieties, but the winter barley cultivar showed significantly more intensive stem growth compared to the spring barley. The spring barley reached all the plant developmental phases significantly earlier than the winter barley. For both cultivars, tillering continued till after first node appearance and there was a definite delay between first node appearance and the beginning of the stem elongation phase. The determination of the full series of phenophases, together with the evaluation of various yield components on the same plant, provide an excellent way of establishing plant developmental patterns and may make a significant contribution to achieving a better understanding of the associations between plant developmental patterns and the adaptation and yielding ability of cereals.

Key words: plant height, leaf number, tiller number, barley

Introduction

Plant developmental patterns and flowering time are some of the most important adaptive characteristics of plants. The genetic regulation of physiological processes acts to ensure that flowering occurs at the seasonal optima for pollination, fertilization and seed development. The development of cereals through their life cycle can be divided into several phases: from

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germination to the vegetative–generative transition in the apex (the vegetative phase coinciding with leaf initiation), from the vegetative–generative transition to stem elongation (spikelet initiation or early reproductive phase), from stem elongation to heading date (spikelet growth or late reproductive phase) and from heading date to physiological maturity (grain set and grain-filling phase) (Borras et al., 2009; McMaster, 2005; Reynolds et al., 2009; Chen et al., 2010). The timing and length of each phase may vary depending on the environmental conditions (mainly temperature and photoperiod), the genotype and their interactions (Borras et al., 2009; Chen et al., 2010) and thus the length of the various developmental phases is an important factor under a given set of ecological conditions in determining the realisable yield potential of a genotype (Gonzales et al., 2005; McMaster, 2005; Chen et al., 2009). The later timing of stem elongation helps to avoid frost damage in early spring; and earlier maturity helps to avoid hot, dry weather during summer. Similarly, the duration between two phases is important in yield component generation. A longer vegetative phase generates more biomass, an extended stem elongation phase is required for an increased number of fertile florets, and a longer grain-filling period may be of benefit in increasing grain weight. The stem elongation phase seems to be especially critical in determining the yield potential of cereals (Reynolds et al., 2009). Extending the duration of this phase without modifying the total time to anthesis has been proposed as a promising breeding tool. A prerequisite for its use is that the duration of the developmental phases before and after stem elongation should be under different genetic control (Borras et al., 2009; Reynolds et al., 2009). Although there is a vast and ever increasing body of knowledge on the genetic determinants of heading date in cereals, much less is known about the genetics of the preceding plant developmental phases and about the genetic regulatory processes of full plant development (Chen et al., 2009; Chen et al., 2010).

The importance of this research field is also underlined by the fact that the changes in local climate conditions caused by global climatic changes cannot be exactly predicted, and neither can their effects on locally adapted plant developmental strategies. A more comprehensive and quantitative understanding of the physiological and genetic determinants of the time to heading and the partitioning of time between the pre-flowering phenophases would allow the fine tuning of adaptation, under both present and future conditions, and the optimisation of development for maximum yield potential. Thus, it is extremely important to characterize the variation existing in various plant developmental phases in cereal germplasm and to identify the determinants which contribute to their genetic control. To study the earlier plant developmental phases, however, especially changes in the structure of the apex and the timing of the stem elongation phase, requires the application of various destructive methods such as dissection of the apex, or the determination of the length of the hollow stem (Gonzales et al., 2005; Chen et al., 2009; Chen et al., 2010), which is a time-

consuming process limiting the sample sizes it is possible to examine. In addition, the destructive nature of these examinations prevents the direct identification of associations between the characteristics of plant developmental phases and yield components.

In this model experiment the major aims were (1) to elaborate a systematic phenotyping procedure for monitoring plant development via the parallel assessment of changes in plant height, leaf and tiller numbers, and (2) to evaluate the data matrices to establish the timing of several consecutive developmental phases and to numerically characterise various developmental parameters. For this purpose, two barley cultivars representing the two major plant growth habit groups (winter and spring) were chosen for phenotyping. The procedure elaborated, together with the statistical analyses, proved to be reliable and effective in studying plant developmental patterns even in a larger set of genotypes.

Materials and methods

After a vernalization treatment of 45 days at +3°C, four plants each of a winter barley cultivar (Kompolti Korai) and a spring barley cultivar (Morex) were grown under controlled environmental conditions with a 16 h photoperiod regime and constant 18°C temperature in the phytotron facilities of the Agricultural Research Institute (HAS), Martonvásár, Hungary using a single Conviron PGR-15 growth chamber (Controlled Environments Limited, Winnipeg, Canada). The plants were regularly measured twice a week for the following parameters: number of leaves on the main stem, attachment height of the last leaf sheath on the main stem and the number of side tillers. The plants were also regularly checked for the appearance of the first node at the base of the main stem [Plant developmental phase 31 (DEV31), Tottman and Makepeace, 1979], and for the appearance of the awns just visible above the last leaf sheath (DEV49). The plants were grown to full maturity and the following yield components were evaluated on each plant: number of reproductive tillers, number of seeds and thousand-kernel weight on the main stem, average number of seeds and thousand-kernel weight on the side tillers, total seed yield per plant.

The statistical analysis was carried out using the Excel for Windows and Statistica 6 software packages. The collected data matrices were evaluated for various characteristics as discussed in the Results section. As the temperature was kept constant throughout the experiment the correlation between chronological (in days) and thermal time (daily mean temperature × day) was unity. The chronological time elapsed from the start of the experiment was applied for regression analysis in the present experiment, but this value could be replaced by thermal time.

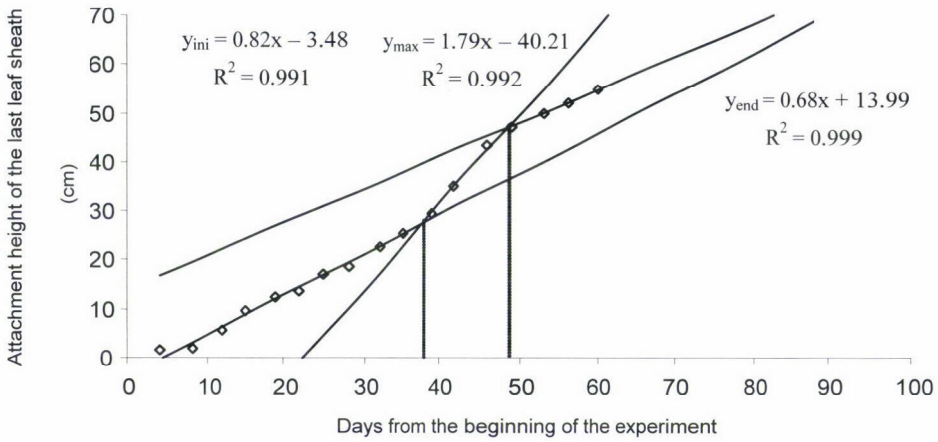
Results

Changes in plant height

In the case of both cultivars, changes in the attachment height of the last leaf sheath on the main stem could be characterized with sigmoid curves, with three clearly contrasting phases (Fig. 1). Slow initial growth was followed by an intensive growing phase, while in the final phase the plant height again changed more slowly. Within the sigmoid curve, these three phases could be well characterised with linear regressions between time and plant height, resulting in

equations for $y_{initial}$, y_{max} and y_{end}, of which y_{max} represents the stem elongation phase. The parameters of the individual equations, such as the steepness of the curve and their intersections, thus represent a numerical characterisation of the growth characteristics of a genotype and as such can be efficiently used in comparing this trait in various cereal genotypes. The intersection of $y_{initial}$ and y_{max} designates the start of the stem elongation phase, coinciding with DEV30 (Tottman and Makepeace, 1979), while that of y_{max} and y_{end} designates the end of the stem elongation phase. The days elapsed between these two points shows the duration of stem elongation.

(a)



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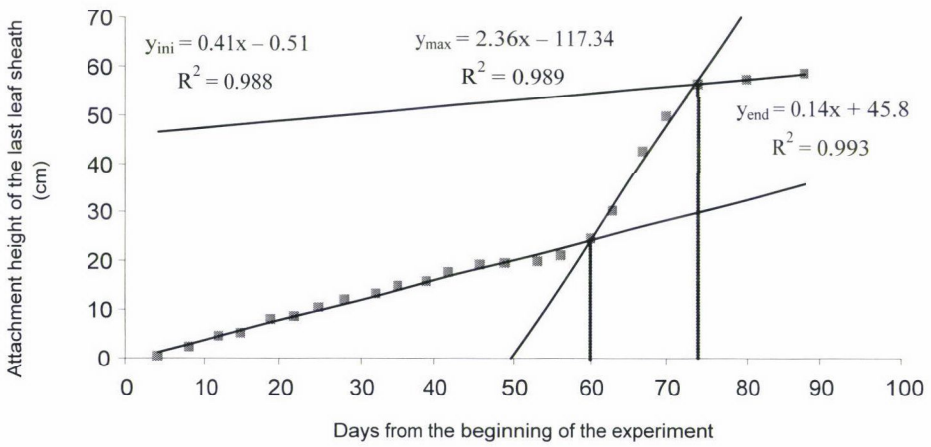

Fig. 1. Changes in the attachment height of the last leaf sheath on the main stem during the experimental period (a) for the spring barley cultivar Morex and (b) for the winter barley cultivar Kompolti Korai

The final plant height of the two cultivars examined was similar in magnitude, being 55 cm for Morex and 58 cm for Kompolti Korai. When the plant growth parameters were taken into account, however, there were significant differences in the growth characteristics of the two genotypes. The initial growth of the spring barley was twice as intensive as that of the winter barley (b_{ini} 0.82 for Morex vs. b_{ini} 0.41 for Kompolti Korai). The length of the stem elongation phase was also similar for the two varieties (11 days for Morex and 14 days for Kompolti Korai), but the winter barley cultivar showed significantly more intensive growth during the stem elongation phase compared to the spring barley (b_{max} being 2.36 and 1.79, respectively). By the end of the stem elongation phase the winter barley reached almost its final height, while the spring barley cultivar continued to grow, though at decreased intensity.

Changes in leaf number on the main stem

The changes in leaf number on the main stem could be well characterized with linear regressions over the course of time for both cultivars (Fig. 2). The final leaf number and the parameters of the equations (y=a+bx) could be used to quantify both the date of final leaf appearance (DEV37), as (final leaf number–0.9)–a)/b, and the date when the final leaf was fully expanded (DEV39), as (final leaf number–a)/b. In addition, the steepness of the curve characterized the rate of leafing out, so the phyllocron for the cultivars could also be evaluated as the reciprocal of the rate of leafing out (Kirby and Appleyard, 1987; Hays and Kirby, 1991; McMaster, 2005).

For the two cultivars examined, there was a significant difference in the final leaf number and in the speed of leafing out. The spring cultivar produced fewer leaves (10), but more rapidly (b= 0.21) than the winter cultivar (13.5 and b=0.17).

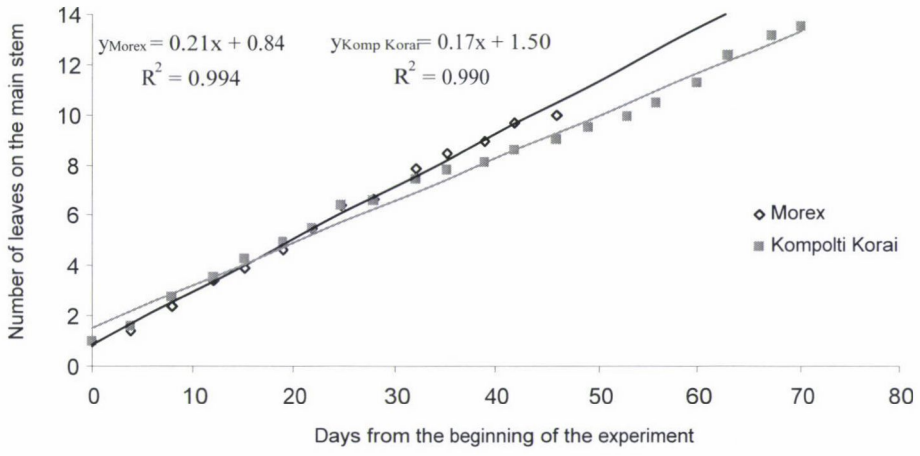

Fig. 2. Changes in the leaf number on the main stem during the time course of the experiment for two barley cultivars (the spring cultivar Morex and the winter cultivar Kompolti Korai)

Changes in tiller number

The changes in tiller number over the course of time could be characterized with a saturation curve, where the maximum tiller number represented the saturation point (Fig. 3). For both cultivars, the phase between the beginning of tillering and the saturation point could be clearly described with linear equations. The following characters could be established using the parameters of these equations: the beginning of tillering, DEV21 = (0–a)/b, the end of tillering, DEV29 = (maximal tiller number in the linear phase – a)/b, the length of the tillering phase, and the intensity of tillering, characterised by the steepness of the equation (b value).

Of the two cultivars, the spring barley produced significantly fewer tillers than the winter cultivar (4.5 vs. 18). This was due partly to its significantly slower tillering (the b values of the two cultivars being 0.18 and 0.48, respectively) and partly to the shorter period of tillering (25 and 38 days, respectively). When the number of reproductive tillers (producing seeds) was taken into account, more than 55% of the developing tillers produced seed in the case of spring barley, while this value was only 19% in the case of winter barley, showing that for the winter barley cultivar most of the tillers produced died without seed set.

Developmental phases

The parameters established via regular observations on changes in plant height, leaf number and tillering during plant development made it possible to quantify the timing of several plant developmental phases. These were the following: beginning of tillering (DEV21), end of tillering (DEV29), appearance

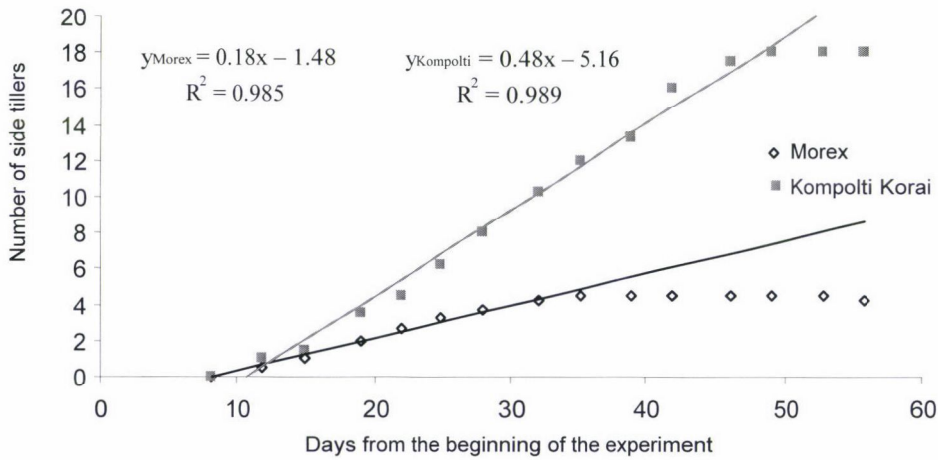

Fig. 3. Changes in the tiller numbers of the two barley cultivars over the time course of the experiment

of the first node at the base of the main stem (DEV31), beginning of the stem elongation phase (DEV30), appearance of the flag leaf (DEV37), flag leaf fully expanded (DEV39), appearance of the awns above the flag leaf sheath (DEV49), end of the stem elongation phase (DEV_SE_end) and achievement of final plant height (DEV_PH_final), where the values in parentheses correspond to the scale described by Tottman and Makepeace (1979). The timing of these plant developmental phases for the two cultivars is given in Table 1.

The spring barley reached all the plant developmental phases significantly earlier than the winter barley, the difference staying relatively the same throughout plant development. Under controlled environmental conditions tillering continued till after first node appearance (DEV31) for both cultivars, but this was more pronounced for the spring cultivar. There was a definite pause between first node appearance and the beginning of the stem elongation phase for both cultivars.

Table 1

Days required to reach a given developmental phase for the two barley cultivars, Morex spring barley and Kompolti Korai winter barley

Cultivar	DEV21	DEV29	DEV31	DEV30	DEV37	DEV39	DEV49	DEV_SEend	DEV_PHfinal
Morex	8	33	21	37	39	45	50	55	60
Kompolti Korai	11	48	45	57	63	67	72	74	77

Discussion

In this model experiment a series of consecutive phenophases covering the life cycle from germination to the end of plant growth (when the plants reached their final height) and several developmental parameters were evaluated in one spring and one winter barley cultivar, via regularly monitoring various plant developmental parameters. The experiment was carried out in a controlled growth chamber with standard environmental conditions (constant long photoperiod, constant temperature), to enhance the elaboration of the methodology. Under these conditions, linear regression was characteristic of the associations between the chronological time and all or most of the time course data of plant height, tiller number and leaf number. These results are in good agreement with previous findings (Robertson et al., 1996; Juskiw et al., 2001; Gonzales et al., 2005; McMaster, 2005; Ghiglione et al., 2008; Borras et al., 2009), underlining the validity of the present methodology. The uniqueness of the present approach is partly due to the parallel monitoring of several developmental traits and to the use of the values of linear regressions in

establishing the exact chronological and/or thermal timing of the consecutive phenophases. The determination of the beginning and end of the intensive stem elongation phase is of special importance. This phenophase is considered to fulfil a central role in determining both adaptation and yield components (Borras et al., 2009; Chen et al., 2009). The frost tolerance of winter cereals remains at a relatively high level before stem elongation starts, even if the vegetative–generative transition has taken place in the apex. In addition, stem elongation provides a more precise representation of the timing of the developmental transition than the heading date, as it occurs closer in time to the transition. Stem elongation is also critical from the aspect of yield, as the number of fertile florets at anthesis is determined during this phase, which in turn determines the final number of grains (Ghiglione et al., 2008; Chen et al., 2009). In some studies, first node appearance was considered to coincide with the stem elongation phase (Borras et al., 2009; McMaster, 2005). The present results, however, show that a relatively long time may elapse between first node appearance and the beginning of stem elongation, and this was more pronounced in the case of spring barley. More detailed experiments involving various genotypes and environments will be required for validating and evaluating the importance of this finding and for studying the associations between the various phenophases and yield.

Yield is the final result of the allocation of resources between vegetative growth, maintenance of the plant structure, and seed set and development. This complex process shows continuous changes throughout the plant life cycle, depending strongly on the specific interaction patterns between the environmental conditions, the developmental phase and the genotype. To dissect the components of such a complex process, one prerequisite is to be able to monitor changes in plant development throughout the plant life cycle. Due to its importance, whole automated plant phenomic platforms have been developed and are used for such monitoring processes (Golzarian et al., 2011). These platforms, however, are very expensive and restrict detailed phenotyping to controlled environmental conditions. The non-destructive phenotyping methodology described here, in spite of its higher labour demand, is cost-effective and efficient for examining plant developmental patterns in larger sample sizes. In addition, it can be standardised and used under a wider range of environments, including phytotron, greenhouse and field-grown experiments. Thus, this method makes it possible to compare the reactions of various genotypes under the same set of environmental conditions, and to examine the effects of various environmental factors on the plant developmental patterns of the same genotypes. In addition, as the plants examined can be grown till maturity, this method is also suitable for analysing direct associations between the length of various plant developmental phases and yielding ability in various types of segregating mapping populations, such as bi-parental mapping populations, near-isogenic lines or multi-parental association mapping populations.

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References

Borras, G., Romagosa, I., van Eeuwijk, F., Slafer, G. A. (2009): Genetic variability in duration of pre-heading phases and relationships with leaf appearance and tillering dynamics in a barley population. *Field Crops Res.*, **113**, 95–104.

Chen, Y., Carver, B. F., Wang, S., Cao, S., Yan, L. (2010): Genetic regulation of developmental phases in winter wheat. *Mol. Breed.*, **26**, 573–582.

Chen, Y., Carver, B. F., Wang, S., Zhang, F., Yan, L. (2009): Genetic loci associated with stem elongation and winter dormancy release in wheat. *Theor. Appl. Genet.*, **118**, 881–889.

Ghiglione, H. O., Gonzalez, F. G., Serrago, R., Maldonado, S. B., Chilcott, C., Cura, J. A., Miralles, D. J., Zhu, T., Casal, J. J. (2008): Autophagy regulated by day length determines the number of fertile florets in wheat. *Plant J.*, **55**, 1010–1024.

Golzarian, M. R., Frick, R. A., Rajendran, K., Berger, B., Roy, S., Tester, M. (2011): Accurate inference of shoot biomass from high-throughput images of cereal plants. *Plant Methods*, **7**, 2.

Gonzales, F. G., Slafer, G. A., Miralles, D. J. (2005): Photoperiod during stem elongation in wheat: is its impact on fertile floret and grain number determination similar to that of radiation? *Functional Plant Biology*, **32**, 181–188.

Hays, R. K. M., Kirby, E. J. M. (1991): Convergence and synchrony – A review of the coordination of development in wheat. *Australian J. Agric. Res.*, **42**, 661–700.

Juskiw, P. E., Jame, Y. W., Kryzanowski, L. (2001): Phenological development of spring barley in a short-season growing area. *Agron. J.*, **93**, 370–379.

Kirby, E. J. M., Appleyard, M. (1987): Development and structure of the wheat plant. pp. 287–311. In: Lupton, F. G. H. (ed.), *Wheat Breeding*, Chapman & Hall, London.

McMaster, G. S. (2005): Phytomers, phyllochrons, phenology and temperate cereal development. *J. Agric. Sci.*, **143**, 137–150.

Reynolds, M., Foulkes, M. J., Slafer, G. A., Berry, P., Parry, M. A. J., Snape, J. W., Angus, W. J. (2009): Raising yield potential in wheat. *J. Exp. Bot.*, **60**, 1899–1918.

Robertson, M. J., Brooking, I. R., Ritchie, J. T. (1996): Temperature response of vernalization in wheat: modelling the effect on the final number of mainstem leaves. *Ann. Bot.*, **78**, 371–381.

Tottman, D. R., Makepeace, R. J. (1979): An explanation of the decimal code for the growth stages of cereals, with illustrations. *Ann. Appl. Biol.*, **93**, 221–234.

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LONG-TERM EFFECT OF FARMYARD MANURE AND MINERAL FERTILISER ON THE YIELD AND YIELD STABILITY OF MAIZE (*Zea mays* L.) IN DRY AND WET YEARS

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The effect of mineral fertilisation, farmyard manure and their combinations on the yield and yield stability of maize was studied in a long-term maize monoculture experiment set up in Martonvásár, Hungary in 1959. The experiment, laid out as a Latin square, included two fertilisation levels [35 t ha^{-1} or 70 t ha^{-1} farmyard manure (FYM) every four years] and seven treatments. The yield results were evaluated using analysis of variance, cumulative yield analysis and stability analysis. The year effect was analysed by dividing the 51 years (1959–2009) into wet (32) and dry (19) years. The rainfall sum for the months Apr.–Sep. averaged 361 mm in the wet years and 232 mm in the dry years.

Among the fertiliser treatments the FYM + mineral fertiliser combination and NPK mineral fertilisation alone gave the highest yields. In more than 50% of the years the higher fertiliser level had no significant yield-increasing effect. The yield differences between the two fertiliser levels were twice as high in wet years as in dry years (0.543 vs. 0.274). Averaged over all seven treatments, the maize yield was 3.959 t ha^{-1} in dry years and 6.250 t ha^{-1} in wet years, giving a yield increment of 2.291 t ha^{-1} in favourable years. Yield stability was greatest when the NPK content of 35 t ha^{-1} FYM was replaced in part (17.5 t ha^{-1} FYM + $N_{1/2}P_{1/2}K_{1/2}$) or in full ($N_1P_1K_1$) by mineral fertiliser, or when 70 t ha^{-1} FYM was applied. Yield stability is an important indicator of the sustainability of crop production.

Key words: long-term experiment, fertilisation effect, maize monoculture, yield stability

Introduction

Long-term agro-ecosystem experiments are large-scale field experiments carried out for more than 20 years in order to study crop yields, nutrient cycles and the effects of the agricultural environment. They provide a source of data for the evaluation of the biological, biogeochemical and environmental dimensions of sustainability and for the validation of models (Rasmussen et al., 1998). Over the last few decades interest in long-term experiments has increased all over the

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world, because it is only from such experiments that satisfactory indicators of cropping sustainability (e.g. yield trends, parameters characteristic of the agro-ecosystem) and of the effects of climate change can be obtained (Leigh and Johnston, 1994; Hillel and Rosenzweig, 2001). Long-term experiments on the agro-ecosystem are underway in many countries and represent the largest databases available in terms of both time and space for the evaluation of changes in the ecosystem. The long-term experiments set up by Béla Győrffy in Martonvásár between 1959 and 1961 are now over 50 years old and can be classified among the classical long-term experiments (Berzsenyi, 2010). A number of reviews have been published on the results achieved in long-term experiments internationally and in Hungary (Leigh and Johnston, 1994; Debreczeni and Debreczeni, 1994; Debreczeni and Németh, 2009; Berzsenyi and Árendás, 2009). In most long-term experiments it is possible to identify cultivation methods capable of sustaining both crop yields and soil quality. Inputs such as organic manure and mineral fertiliser lead to an increase not only in crop yields, but also in the quantity of crop residues. Recycling larger residue quantities into the soil has a steadily increasing positive effect on the soil C content. In this way numerous soils that were previously sources of atmospheric CO_2 have been transformed into CO_2 consumers, which is of especial importance from the point of view of climate change. If the return of plant residues to the soil is hindered by economic considerations, this is likely to result in a deterioration in soil quality and the loss of sustainability (Rasmussen et al., 1998).

A combination of mineral fertiliser and farmyard manure leads to the highest yields in many parts of the world. It has been reported that the optimum combination of organic and inorganic fertilisers led to a 7% increase in crop yields in the continental regions of Europe, compared to the application of mineral fertiliser alone (Powlson et al., 1996). In earlier studies (Berzsenyi et al., 2000; Árendás et al., 2010) it was established that the joint application of organic and mineral fertiliser was an efficient way of fertilising maize and wheat, providing favourable conditions for the manifestation of the rotation effect. In experiments in Hungary the yield-enhancing effect of farmyard manure was found to be greatly dependent on soil properties and environmental factors. In some long-term experiments (e.g. Sarkadi, 1994) the application of farmyard manure every four years had a yield-enhancing effect equivalent to around two-thirds of that of mineral fertiliser with the same NPK content.

One important indicator of sustainability is yield stability (Piepho, 1998). The measurement of yield stability over time consists of several components: (i) the correlation between the yield and the local environment, (ii) the mean yield level and (iii) the yield variability (Mead et al., 1986). A stable system can be defined as one which changes little in response to changes in the environment. Yield trends over time in long-term experiments are reliable indicators of the sustainability of the production system (Jones and Singh, 1999).

In general, most long-term experiments can identify management practices capable of maintaining crop yield and soil quality. In the long-term maize monoculture experiment set up in Martonvásár in 1959, studies were made on the effect of mineral fertilisation, farmyard manure and their combinations on the yield and yield stability of maize. The question originally was, whether half or all of the NPK contents in 35 or 70 t ha^{-1} farmyard manure, applied every four years, could be replaced by mineral NPK fertiliser. Studying the effect of the year is particularly important when attempting to predict the expected effects of climate change (Hillel and Rosenzweig, 2011).

Materials and methods

The long-term fertilisation experiment was set up by Béla Győrffy and his colleagues as a maize monoculture in the institute nursery in Martonvásár, Hungary (N 47°21', E 18°49') in 1959. The ploughed layer was a slightly acidic humus-containing loam (chernozem with forest residues) with poor supplies of phosphorus and good supplies of potassium.

Experimental treatments

The experiment was laid out as a Latin square, with seven treatments and seven replications. The plot size was 80 m^2. The experiment included two fertiliser levels: the NPK active ingredient contents of (1) 35 t ha^{-1} or (2) 70 t ha^{-1} farmyard manure (FYM), applied in the form of (a) FYM, (b) FYM + mineral fertiliser or (c) mineral fertiliser. The treatments were as follows:

1. Control, without fertilisation
2. 35 t ha^{-1} FYM every four years
3. 17.5 t ha^{-1} FYM every four years + NPK mineral fertiliser supplementation ($N_{1/2}P_{1/2}K_{1/2}$)
4. NPK mineral fertiliser equivalent to the active ingredients of 35 t ha^{-1} FYM ($N_1P_1K_1$)
5. 70 t ha^{-1} FYM every four years
6. 35 t ha^{-1} FYM every four years + NPK mineral fertiliser supplementation ($N_1P_1K_1$)
7. NPK mineral fertiliser equivalent to the active ingredients of 70 t ha^{-1} FYM ($N_2P_2K_2$)

The P and K fertilisers were applied once every four years, generally at the same time as FYM application, while the N fertiliser was divided into four yearly amounts. Averaged over the cycles, the active ingredients applied each year in each treatment were as follows (kg ha^{-1}): Treatments 2–4: N: 66, P_2O_5: 38, K_2O: 75, Treatments 5–7: N: 132, P_2O_5: 76, K_2O: 150.

Analysis of variance

The effect of the treatments was first evaluated separately for each year using analysis of variance for a Latin square design. The Latin square model is the following:

$$Y_{ij} = \mu + r_i + c_j + t_{k(ij)} + \varepsilon_{ij}$$

where: Y_{ij}: observed yield, μ: overall mean, r_i: row effect, c_j: column effect, $t_{k(ij)}$: treatment variation, ε_{ij}: random unit variation.

In the next step the F values were calculated for comparisons between and within treatment groups. Five treatment contrasts were considered: (i) between control and fertilised treatments [(T_1) vs. (T_2, T_3, T_4, T_5, T_6, T_7)], (ii) between the two fertilisation levels [(T_2, T_3, T_4) vs. (T_5, T_6, T_7)], (iii) between the two levels of FYM (T_2 vs. T_5), (iv) between the two levels of FYM + mineral fertiliser (T_3 vs. T_6) and (v) between the two levels of mineral fertiliser (T_4 vs. T_7).

Finally the experimental data for 51 years were evaluated together and for types of years. The treatment structure was year × treatment, while the block structure was row × column. To analyse the effect of the weather, the 51 years (1959–2009) were divided into wet (32) and dry

(19) years on the basis of differences in the rainfall quantity during the vegetation period (Apr.–Sep.). The mean rainfall for this period was 361 mm in the wet years and 232 mm in the dry years.

Cumulative yield analysis

This method involves calculating the yield differences each year between the individual treatments and a basic treatment and cumulating these differences each year. Cumulative yield differences demonstrate the total yield difference in any year between a given treatment and the basic treatment (Sváb, 1981). Treatment 2 (35 t ha^{-1} FYM every four years) was taken as the basic treatment.

The random coefficient regression method (Payne et al., 2010) of the REML linear mixed model was chosen for the statistical analysis of cumulative yield differences. This method simultaneously models changes over time in the responses to each treatment by fitting linear, quadratic or polynomial functions to the data. The analysis provides SED (standard error of differences) and estimated LSD (least significant differences) values for the whole period and for every year.

Stability analysis

A common approach to stability analysis is to regress the performance of the system onto an environmental index computed as the mean of all observations in an environment. This index may be taken as a measure of the productivity of the environment. The regression techniques used to develop stability parameters are based on linear slope and deviation from that slope. The regression approach was first suggested by Yates and Cochran (1938), followed later by Finlay and Wilkinson (1963) and Eberhart and Russell (1966).

Among the multivariate methods of stability analysis the AMMI (additive main effects and multiplicative interaction) model was used, which is a combination of analysis of variance (ANOVA) and principal component analysis (PCA) (Crossa, 1990). In the first part of the AMMI analysis, ANOVA is used to dissect the total variance into orthogonal sources: genotype (G), environment (E) and genotype × environment interaction (G×E), while in the second phase PCA is applied to dissect the G×E interaction into a number of orthogonal principal component variables (PCA axes). The greater the value of the principal component, the greater the contribution of the treatment to the interaction, i.e. the smaller the yield stability.

Results

Annual effect of fertilisation treatments and treatment groups

Based on the results of ANOVA on the annual yield data in the long-term experiment, the significance of the F values was calculated for the treatments and treatment groups (Table 1). With the exception of two years the effect of the treatments was significant. One of the non-significant cases was 1959, the year the experiment was set up, and the other was 1990, a year when there was a very severe drought. The significance of the F values for the difference in yield between the unfertilised control and the fertilised treatments (contrast 1) was similar to the annual results for the experimental treatments. The effect of the fertiliser treatments was significant in 94% of the years. Averaged over the fertilised treatments the yield was 2.08 t ha^{-1} higher than in the control treatment (5.693 vs. 3.613 t ha^{-1}). The difference in yield between the control and the fertilised treatments was smaller in dry years (1.73 t ha^{-1}) than in wet years (2.289 t ha^{-1}). There was a significant difference in the maize yields of the two

fertiliser levels (contrast 2) in 32 years. In 27 years the yield for the higher fertiliser level was significantly greater than for the lower level, while the yield for the lower level was only significantly higher in five years (in dry years). Averaged over the 51 years the yield at the higher fertiliser level was only 0.44 t ha^{-1} greater than for the lower level. For the different forms of fertiliser, this yield increase in favour of the higher fertiliser level, averaged over 51 years, was 0.553 t ha^{-1} for FYM, 0.35 t ha^{-1} for FYM + mineral fertiliser and 0.424 t ha^{-1} for mineral fertiliser. The yield differences between the two levels of FYM (contrast 3), the two levels of FYM + mineral fertiliser (contrast 4) and the two levels of mineral fertiliser (contrast 5) were not significant in 62.7%, 51% and 64.7% of the years, respectively.

Analysis of fertiliser effects using cumulative yield analysis

The long-term effect of FYM and mineral fertiliser in a maize monoculture in terms of cumulative yield differences compared to a basic treatment (35 t ha^{-1} FYM every four years) is illustrated in Figure 1. The REML analysis revealed very significant ($P<0.001$) treatment and year effects and treatment × year interactions (Table 2). The changes in treatment effects over time were described by a quadratic model (Fig. 1).

It is clear from the steeply declining curve that the yield in the unfertilised control decreased to a greater extent every year. By the 51st year, the total yield deficiency was 72.9 t ha^{-1} compared to the basic treatment and 129.8 t ha^{-1} compared with treatment 7, which gave the best results.

There was no difference between the fertiliser treatments in the first seven years and very little over the first 10 years. Real differences began to appear after the 14th year, providing a clear proof of the fact that fertilisation experiments only provide valuable data if they are continued over several decades.

Only the yield obtained in treatment 7 (NPK fertiliser equivalent to 70 t ha^{-1} FYM) was distinctly higher than that in the other treatments by the 12th year, gradually increasing its lead in later years. Treatment 6, i.e. the basic quantity of FYM + NPK supplementation, gradually proved to be more effective than the 70 t ha^{-1} rate of FYM after the 18th year. Treatments 3–5 gradually fell behind treatments 6–7, though initially there was very little difference between them. Over the last 20 years, however, the effect of treatment 4 became more pronounced. In the 51st year the cumulative yield increases compared to the basic treatment were as follows (t ha^{-1}): treatment 3: 31.6, treatment 4: 38.6, treatment 5: 26.9, treatment 6: 49.5, treatment 7: 56.9.

Table 1

Significance levels of the F values of treatments and treatment contrasts in the years 1959–2009

Contrasts	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975
	Significance of F-values																
Treatments	NS	*	*	*	***	***	***	***	***	**	***	***	***	***	***	***	***
Contrast 1	NS	***	**	NS	***	***	***	***	***	*	***	***	***	***	***	***	***
Contrast 2	NS	NS	NS	**	NS	NS	***	***	NS	***	***	***	NS	***	NS	*	***
Contrast 3	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	***	NS	*	NS	*	***
Contrast 4	NS	NS	NS	***	NS	NS	***	*	NS	**	***	***	NS	*	NS	NS	***
Contrast 5	NS	NS	NS	NS	NS	NS	*	**	NS	NS	***	***	NS	NS	NS	NS	***

Contrasts	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
	Significance of F-values																
Treatments	***	***	***	***	***	***	***	***	***	***	***	***	***	***	NS	***	***
Contrast 1	***	***	***	***	***	***	***	***	***	***	***	***	***	***	NS	***	***
Contrast 2	NS	***	***	**	**	**	***	NS	NS	***	***	*	NS	***	NS	NS	**
Contrast 3	NS	***	***	NS	*	NS	*	NS	NS	***	***	NS	NS	***	NS	NS	NS
Contrast 4	NS	*	***	NS	NS	*	NS	NS	NS	***	**	NS	**	***	NS	NS	**
Contrast 5	NS	***	***	NS	NS	NS	*	NS	NS	**	***	NS	***	***	NS	NS	NS

Contrasts	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
	Significance of F-values																
Treatments	***	***	***	***	***	***	***	***	***	***	**	***	***	***	***	***	***
Contrast 1	***	***	***	***	***	***	***	***	***	***	***	***	***	***	*	***	***
Contrast 2	**	NS	NS	***	***	NS	***	**	*	*	*	NS	***	*	**	*	NS
Contrast 3	NS	NS	NS	*	***	NS	*	**	**	NS	NS	**	*	NS	NS	NS	NS
Contrast 4	**	NS	NS	**	***	*	*	NS	NS	**	*	NS	***	NS	**	NS	NS
Contrast 5	NS	NS	NS	***	NS	NS	***	NS	NS	*	NS	NS	***	**	**	NS	NS

Contrast 1: control vs. fertilisation treatments; contrast 2: two levels of fertilisation; contrast 3: two levels of farmyard manure; contrast 4: two levels of organic+mineral fertilisation; contrast 5: two levels of mineral fertilisation. *** Significant at $P \leq 0.001$, ** Significant at $P \leq 0.01$, * Significant at $P \leq 0.05$, ^{NS} Non-significant at $P \leq 0.05$.

Table 2

Analysis of cumulative yield differences using the REML random coefficient regression method

Fixed term	Wald statistics	n.d.f.	F statistic	d.d.f.	F probability
Treatment	55192	5	11039	288	< 0.001
Time	6647	1	6647	288	< 0.001
Time square	283	1	283	288	< 0.001
Treatment × Time	28089	5	5618	288	< 0.001
Treatment × Time square	182	5	36.5	288	< 0.001

n.d.f.: numerator degrees of freedom; d.d.f.: denominator degrees of freedom

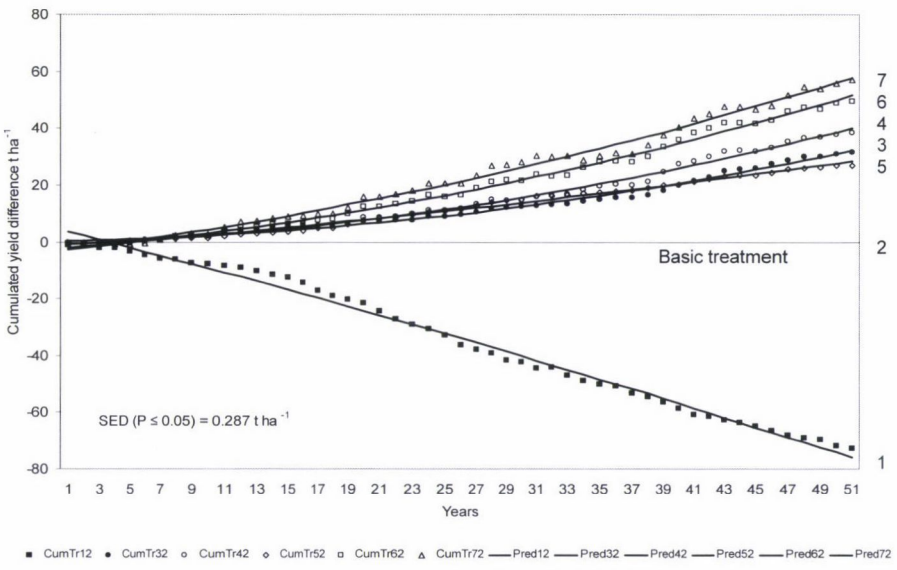

Fig. 1. Long-term effect of FYM and mineral fertilisation on the cumulated yield differences compared to a basic treatment. The equations of the curves are: Tr1.2, $y = 5.015 - 1.393x - 0.00381x^2$ ($R^2 = 0.991$); Tr3.2, $y = 0.297 + 0.0918 + 0.0104x^2$ ($R^2 = 0.989$); Tr4.2, $y = -0.925 + 0.190x + 0.012x^2$ ($R^2 = 0.993$); Tr5.2, $y = -2.416 + 0.452x + 0.00295x^2$ ($R^2 = 0.990$); Tr6.2, $y = -2.762 + 0.465x + 0.0118x^2$ ($R^2 = 0.994$); Tr7.2, $y = -3.518 + 0.690x + 0.0100x^2$ ($R^2 = 0.991$). Cum: measured values of yield; Pred: predicted yield

Main effects and interaction of fertilisation and year

The effects of the seven different fertiliser treatments on a maize monoculture were first evaluated on the data of all 51 years by means of analysis of variance (Table 3). The effects of treatments and years and the treatment × year interaction all proved to be significant. The results of ANOVA clearly indicated the importance of the year effect (treatment MS = 269.7, year MS = 112.4), so in the next step the evaluation was continued separately for dry (19) and wet (32) years. It is clear from the ANOVA results presented in Table 3 that the main effects of the treatment and the environment (year) were of similar magnitude in the dry years (treatment MS = 67.6, year MS = 55.2), while in wet years there was a pronounced increase in the effect of the treatments, which became more than four times as great as the year effect (treatment MS = 208.6, year MS = 50.3). The coefficient of variance was almost twice as high in dry years as in wet years (19% vs. 10.6%).

The analysis of variance on 51 years of data (Fig. 2a) clearly demonstrated that the maize yield was highest at the higher level of fertilisation, irrespective of whether it was applied in the form of NPK fertiliser or as a combination of NPK and FYM (treatments 6 and 7). The yields in treatments 3–5 were not significantly different, indicating that the effect of 70 t ha^{-1} FYM on

the yield was no greater than that of the $N_1P_1K_1$ (treatment 4), even if half the latter was applied in the form of FYM (treatment 3). The effect of 35 t ha^{-1} FYM every four years (treatment 2) on the yield was significantly smaller than that of the other fertiliser treatments.

The grouping of the 51 years of the long-term experiment as wet and dry years gave a clear picture of the very substantial effect of the year on the yield. The effect of FYM or the equivalent NPK content of mineral fertiliser on the maize yield in dry (19) and wet (32) years is illustrated in Figure 2b. Averaged over the seven treatments, the maize yield was 3.959 t ha^{-1} in dry years and 6.250 t ha^{-1} in wet years, i.e. the yield increment in favourable years was 2.291 t ha^{-1}. In both dry and wet years there was a significant difference ($P \leq 0.05$ and $P \leq 0.001$, respectively) between the two fertilisation levels, but the yield difference was twice as great in wet years as in dry years (0.543 vs. 0.274). In dry years there was also a significant difference ($P \leq 0.05$) between the two levels of FYM (0.47 t ha^{-1}). In wet years the mixed form of fertilisation gave higher yields than FYM alone at both fertilisation levels, while in dry years the difference was only significant at the lower level ($P \leq 0.05$). The yield-increasing effect of a combination of FYM and NPK mineral fertiliser did not differ significantly from that of NPK mineral fertiliser alone either in the joint analysis of the 51 years or in the separate analysis of years or fertilisation levels.

Table 3
Analysis of variance on the treatment and year effects in all the years, and for dry and wet years separately

Source of variation	Joint evaluation (51 years)		Wet years (32 years)		Dry years (19 years)	
	d.f.	MS	d.f.	MS	d.f.	MS
Row	6	34.8	6	16.8	6	19.5
Column	6	82.8	6	60.7	6	23.2
Treatments	6	269.7***	6	208.6***	6	67.6***
Contrast 1	1	1324.3***	1	1005.8***	1	340.9***
Contrast 2	1	104.8***	1	98.8***	1	15.0**
Contrast 3	1	54.6***	1	40.6***	1	14.7**
Contrast 4	1	21.8*	1	24.1***	1	1.63^{NS}
Contrast 5	1	32.2**	1	35.2***	1	2.53^{NS}
Residual	30	3.03	30	1.95	30	1.54
Year	50	112.4***	31	50.3***	18	55.2***
Year × Treatment	300	2.96***	186	2.53***	108	3.50***
Residual	2100	0.49	1302	0.44	7.56	0.57
CV%	13.0		10.6		19.0	

Contrast 1: control vs. fertilisation treatments; contrast 2: two levels of fertilisation; contrast 3: two levels of farmyard manure; contrast 4: two levels of organic + mineral fertilisation; contrast 5: two levels of mineral fertilisation. *** Significant at $P \leq 0.001$, ** Significant at $P \leq 0.01$, *Significant at $P \leq 0.05$, ^{NS} Non-significant at $P < 0.05$.

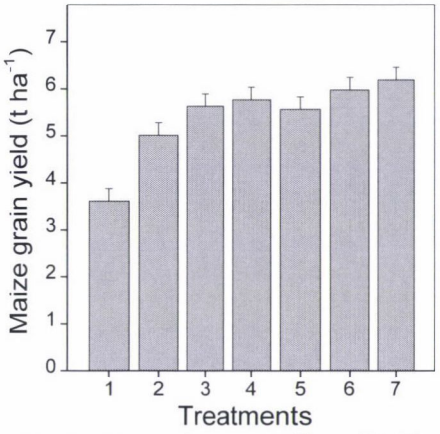

Fig. 2a. 51-year long-term effect of fertiliser treatments on the yield of maize. Error bars: LSD at P=0.05

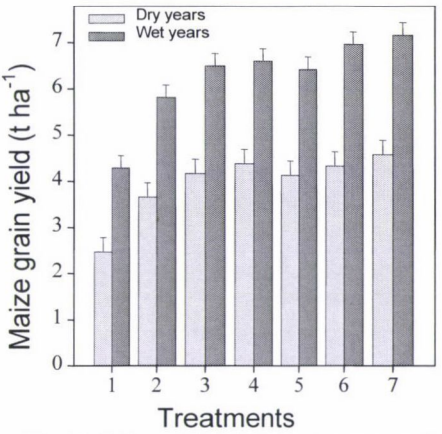

Fig. 2b. Effect of fertiliser treatments on the yield of maize in dry (19) and wet (32) years. Error bars: LSD at P=0.05

Stability of maize yields in various treatments and years

The effects of the various fertilisation treatments on maize yield stability are illustrated in Figure 3 on the basis of the Finlay and Wilkinson (1963) model. The linear function gave a good fit to the data. In all cases the *t*-test on the regression coefficient (b) was significant at the 0.1% level.

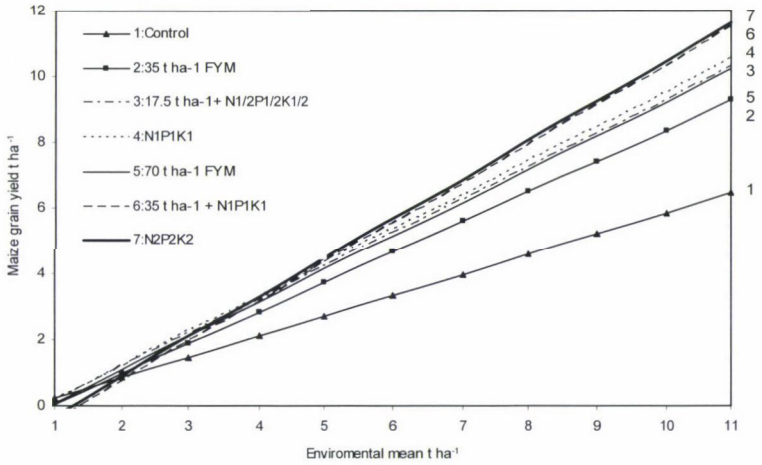

Fig. 3. Effect of FYM and mineral fertiliser on maize yield stability (1959–2009). The equations of the lines are: 1, $y = 0.222 + 0.625x$ ($R^2 = 0.63$); 2, $y = 0.048 + 0.923x$ ($R^2 = 0.87$); 3, $y = 0.190 + 1.012x$ ($R^2 = 0.969$); 4, $y = 0.194 + 1.036x$ ($R^2 = 0.939$); 5, $y = 0.084 + 1.014x$ ($R^2 = 0.937$); 6, $y = -0.444 + 1.195x$ ($R^2 = 0.907$); 7, $y = -0.295 + 1.195x$ ($R^2 = 0.837$)

It can be clearly seen in Figure 3 that the unfertilised control (treatment 1) had low values of both yield level and yield stability (b = 0.625). Treatments 3–5, when the 35 t ha^{-1} rate of FYM was replaced in part or in full by mineral fertiliser or when the 70 t ha^{-1} rate of FYM was applied, had average stability (b values between 1.012 and 1.036). Treatment 2 (35 t ha^{-1} FYM) was better adapted to an unfavourable environment, while treatments 6 and 7 (35 t ha^{-1} FYM + $N_1P_1K_1$ and $N_2P_2K_2$) had better adaptability to a favourable environment.

The AMMI analysis revealed that the genotype and environment effects and the G × E interaction were all strongly significant ($P < 0.01$), while their percentage share of the treatment combination SS values was 19.9, 68.2 and 10.9%, respectively (Table 4). The separation of the period examined into wet and dry years revealed an increase in the share of the treatment in SS in wet years and an increase in that of the environment and the interaction in dry years. The effect of the environment was found to be dominant in all three data groups.

The effect of the treatments on the variability in maize yields is illustrated in Figure 4 on the basis of multivariate stability analysis (AMMI). The yield averages for the seven fertiliser treatments (G1–G7) and the 51 environments (E1–E51) are shown on the X axis and the principle component values of the interaction on the Y axis. The PCA1 values explained 71% of the interaction SS (Table 4). As can be seen in Figure 4, the control (G1) and the G6 (35 t ha^{-1} FYM + $N_1P_1K_1$) and G7 ($N_2P_2K_2$) treatments made the greatest contributions to the interaction, while the G3 (17.5 t ha^{-1} FYM + $N_{1/2}P_{1/2}K_{1/2}$), G5 (70 t ha^{-1} FYM) and G4 ($N_1P_1K_1$) treatments had the greatest yield stability. Figure 4 also clearly illustrates the grouping of the environments (years). Dry years with 241 mm rainfall and low yields (3.19 t ha^{-1}) are to be found in the lower left quadrant of the coordinate system and those with 246 mm rainfall and below-average yields (4.33 t ha^{-1}) in the upper left quadrant. On the right of the coordinate system, on the other hand, years in which the rainfall supplies were favourable (354 mm) are found, with PCA values between 0 and ±0.5 and stable, high yields (7.39 t ha^{-1}).

Table 4

Additive main effects and multiplicative interaction analysis of variance for the whole period (51 years) and in wet (32) and dry (19) years

Source of variation	Whole period			Wet years			Dry years		
	d.f.	SS	MS	d.f.	SS	MS	d.f.	SS	MS
Treatment combinations	356	8126	22.8***	223	3281	14.71***	132	1778	13.47***
Genotypes (G)	6	1618	269.8***	6	1252	208.6***	6	406	67.6***
Environments (E)	50	5620	112.4***	31	1560	50.3***	18	994	55.2***
G × E	300	887	2.96***	186	470	2.53***	108	378	3.50***
Interaction PCA1	55	631	11.48***	36	308	8.56***	23	294	12.76***
Interaction PCA2	53	137	2.59***	34	95	2.80***	21	38	1.82***
Residuals	192	118	0.62^{NS}	116	67	0.58^{NS}	64	47	0.73^{NS}
Error	1836	1406	0.77	1152	867	0.49	684	539	0.79

*** Significant at $P \leq 0.001$, ^{NS} Non-significant at $P < 0.05$.

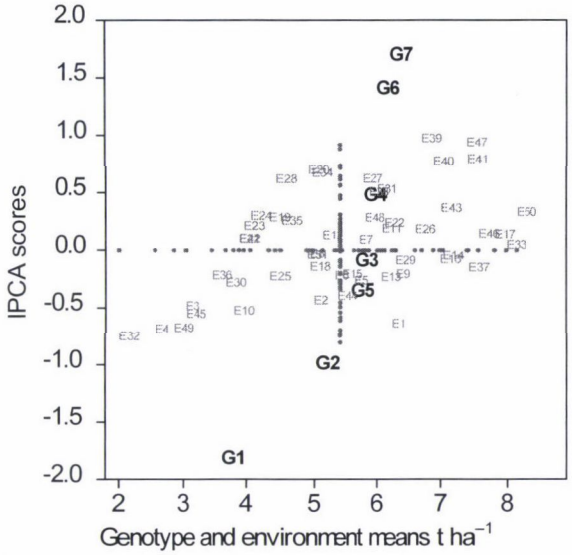

Fig. 4. AMMI diagram of the average yield and 1st principal values of the 7 fertilisation treatments (G1–G7) and the 51 environments (E1–E51)

Discussion

A comparison of the effects of various fertiliser treatments based on the data recorded in the long-term experiment between 1959 and 2009 revealed that the significantly highest maize yields were obtained at high rates of NPK or NPK + FYM combinations. The yield-increasing effect of a combination of FYM and NPK mineral fertiliser did not differ significantly from that of NPK mineral fertiliser alone either in the joint analysis of the 51 years or in the separate analysis of years or fertilisation levels. These results confirm those of Powlson et al. (1996), who found that a combination of inorganic and organic fertilisers gave the highest yields in many parts of the world. In previous studies (Berzsenyi et al., 2000) it was established that the joint application of organic manure and mineral fertiliser was an efficient way of fertilising maize and wheat and also created favourable conditions for the manifestation of the rotation effect. Manuring adds not only nutrients but also organic matter, which can be thought of as an ecological method of sustaining soil productivity. The long-term application of supplemental C sources such as farmyard manure or additional crop residues may increase soil aggregate stability, microbial and earthworm activity and soil water storage, and thus optimise the soil physical environment for crop growth (Karlen and Doran, 1993).

Analysis of variance on the annual data from the long-term experiment showed that in 43% of the years the higher rate of fertilisation did not significantly influence the maize yield. This is important from both the economic

and environmental point of view and draws attention to the danger of over-fertilisation in the given ecological environment and to the need to apply environment-friendly, cost-saving fertilisation methods (Árendás and Csathó, 2002).

The influence of the year on the yield is demonstrated by the fact that in dry years the average yield reduction (2.291 t ha^{-1}) exceeded the yield increment attributable to fertilisation (2.08 t ha^{-1}). In dry years not only was the yield lower, but the effect of the fertilisation treatments also decreased substantially compared with that of the environment. The effect of the higher fertiliser level was not significant in dry years, so the lower rate should be applied. In earlier studies (Berzsenyi and Győrffy, 1997) it was shown that lower levels of fertilisation had greater stability. Recent research revealed a significant reduction in the dry matter accumulation per plant and in the size and growth rate of the leaf area in maize in dry years, compared with wet years (Berzsenyi et al., 2011; Micskei et al., 2010).

Yield stability is an important characteristic when judging the value of a cropping system relative to others (Piepho, 1998). Yield stability depends on yield components and other characteristics, such as resistance to pests and tolerance to environmental stress factors (Kang, 1998).

Fertiliser recommendations could possibly be refined through the use of stability analysis when assessing agronomic treatment responses over time. Both methods of stability analysis indicated that the yield stability was greatest when the NPK content of 35 t ha^{-1} FYM were replaced in part (17.5 t ha^{-1} FYM + $N_{1/2}P_{1/2}K_{1/2}$) or in full ($N_1P_1K_1$) by mineral fertiliser, or when 70 t ha^{-1} FYM was applied. As issues of sustainability become increasingly important, stability analysis may assist in the understanding of yield as a function of the environment. It can be concluded that the effect of fertilisation and year on the magnitude and stability of maize yields can only be measured in long-term experiments and by comparing the results achieved with various analytical methods.

References

Árendás, T., Bónis, P., Csathó, P., Molnár, D., Berzsenyi, Z. (2010): Fertiliser responses of maize and winter wheat as a function of year and forecrop. *Acta Agron. Hung.*, **58** (Suppl.)., 109–114.

Árendás, T., Csathó, P. (2002): Comparison of the effect of equivalent nutrients given in the form of farmyard manure or fertilisers in Hungarian long-term field trials. *Commun. Soil Sci. Plant Anal.*, **30**, 2861–2878.

Berzsenyi, Z. (2010): Significance of 50-year-old long-term experiments in Martonvásár in improving crop production. *Acta Agron. Hung.*, **58** (Suppl.), 23–34.

Berzsenyi, Z., Árendás, T. (eds.) (2009): *Tartamkísérletek jelentősége a növénytermesztés fejlesztésében.* (Significance of long-term experiments in improving crop production.) Jubileumi tudományos konferencia. (Jubilee Scientific Conference.) Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár.

Berzsenyi, Z., Árendás, T., Bónis, P., Micskei, G. (2011): Long-term effect of crop production factors on maize productivity in different years. pp. 374–377. *Climate Change: Challenges and Opportunities in Agriculture.* AGRISAFE Final Conference. Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár.

Berzsenyi, Z., Győrffy, B. (1997): Az istállótrágya és a műtrágya hatása a kukorica (*Zea mays* L.) termésére és terméstabilitására monokultúra tartamkísérletben. [Effect of stable manure and mineral fertiliser on the yield and yield stability of maize (*Zea mays* L.) in a long-term monoculture experiment.] *Növénytermelés*, **46**, 509–527.

Berzsenyi, Z., Győrffy, B., Lap, D. Q. (2000): Effect of crop rotation and fertilisation on maize and wheat yields and yield stability in a long-term experiment. *Europ. J. Agron.*, **13**, 225–244.

Crossa, J. (1990): Statistical analyses of multilocation trials. *Adv. Agron.*, **44**, 55–85.

Debreczeni, B., Debreczeni, B. (1994): *Trágyázási kutatások 1960–1990.* (Fertilisation experiments 1960–1990). Akadémiai Kiadó, Budapest.

Debreczeni, B., Németh, T. (2009): *Az országos műtrágyázási tartamkísérletek (OMTK) kutatási eredményei (1967–2001).* [Results of the national long-term fertilisation experiments (1967–2001)]. Akadémiai Kiadó, Budapest.

Eberhart, S. A., Russell, W. A. (1966): Stability parameters for comparing varieties. *Crop Sci.*, **6**, 36–40.

Finlay, K. W., Wilkinson, G. N. (1963): The analysis of adaptation in a plant breeding programme. *Aust. J. Agric. Res.*, **14**, 742–754.

Hillel, D., Rosenzweig, C. (2011): *Handbook of Climate Change and Agroecosystems.* Imperial College Press, London.

Jones, M. J., Singh, M. (1999): Time trends in crop yields in long-term trials. *Expl. Agric.*, **36**, 165–179.

Kang, M. S. (1998): Using genotype-by-environment interaction for cultivar development. *Adv. Agron.*, **62**, 199–252.

Karlen, D. L., Doran, J. W. (1993): Agroecosystem responses to alternative crop and soil management systems in the U.S. corn-soybean belt. *International Crop Science I.* Crop Science Society of America, Madison, pp. 55–61.

Leigh, R. A., Johnston, A. E. (1994): *Long-term Experiments in Agricultural and Ecological Sciences.* CAB International, Wallingford.

Mead, R., Riley, J., Dear, K., Singh, S. P. (1986): Stability comparison of intercropping and monocropping systems. *Biometrics,* **42**, 253–266.

Micskei, G., Jócsák, I., Berzsenyi, Z. (2010): Effect of farmyard manure and mineral fertiliser on the growth of maize (*Zea mays* L.) in a long-term experiment. II. Using the Hunt-Parsons program for plant growth analysis. *Acta Agron. Hung.*, **58**, 355–365.

Payne, R., Welham, S., Harding, S. (2010): *A Guide to REML in GenStat.* VSN International, Hertfordshire.

Piepho, H. P. (1998): Methods for comparing the yield stability of cropping systems – a review. *J. Agron. Crop Sci.*, **180**, 193–213.

Powlson, D. S., Smith, P., Smith, J. U. (1996): *Evaluation of Soil Organic Matter Models Using Existing Long-Term Datasets.* Springer-Verlag, Berlin.

Rasmussen, P. E., Goulding, K. W. T., Brown, J. R., Grace, P. R., Janzen, H. H, Körshens, M. (1998): Long-term agroecosystem experiments: assessing agricultural sustainability and global change. *Science*, **282**, 893–896.

Sarkadi, J. (1994): Szerves- és műtrágyák hatása a búza és kukorica termésére erdőmaradványos csernozjomon. (Effect of organic and inorganic fertilisers on the yield of wheat and maize on chernozem soil with forest residues.) pp. 315–322. In: Debreczeni, B., Debreczeni, B. (eds.), *Trágyázási kutatások 1960–1990.* (Fertilisation experiments 1960–1990). Akadémiai Kiadó, Budapest.

Sváb, J. (1981): *Biometriai módszerek a kutatásban.* (Biometric methods in research.) Mezőgazdasági Kiadó, Budapest.

Yates, F., Cochran, W. G. (1938): The analysis of a group of experiments. J. Agric. Sci., **28**, 556–580.

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EFFECT OF NPK ENRICHMENT ON GROWTH, YIELD AND QUALITY TRAITS IN RICE BEAN (*Vigna umbellata*)

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The genotypic response of the growth, yield and quality traits of rice bean [*Vigna umbellata* (Thunb.) Ohwi and Ohashi] to NPK enrichment was studied in relation to different fertilizer treatments. The treatments consisted of three NPK levels, namely 0:0:0 (T_0) control, 10:30:10 (T_1) and 20:60:20 (T_2) kg/ha. There were significant differences in plant height 115 days after sowing, days to maturity and tryptophan content (g/16 g N) in various rice bean genotypes with different fertilizer levels. Higher seed yields were recorded for the genotypes JCR-20(S), IC-140796, IC-019352 and JCR-152 as compared to the check variety (BRS-2). The fertilizer treatments significantly affected growth, yield and its contributing traits: plant height, number of pod clusters/plant, seeds/pod, seed yield/plot and total pods picked at maturity. Little variation was observed between the fertilizer treatments for the crude protein (%) and methionine (g/16 g N) contents, but significant variation in the tryptophan content was detected for genotype LRB-40-2. Seed yield and its contributing traits responded positively to the fertilizer treatments. Among the three fertilizer treatments tested in the experiment, the T_2 treatment was found to be promising for increasing seed yield. Thus, it can be concluded from the study that the rice bean crop is responsive to fertilizers and that the application of optimal levels of NPK could enhance its productivity.

Key words: rice bean, fertilizer treatment, NPK enrichment, quality traits, yield parameters, tryptophan

Introduction

Rice bean [*Vigna umbellata* (Thumb.) Ohwi and Ohashi] has recently been identified as a promising pulse crop. It has high amounts of protein (18–25%) and limiting amino acids such as tryptophan and methionine. It also has higher amounts of vitamins (riboflavin and thiamine) and minerals than other pulses (Singh et al., 1980). Although a native of South and South East Asia, rice bean is grown in various parts of the world. It grows well in climates ranging

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from sub-tropical to temperate. In Asia rice bean cultivation is common in India, Burma, China, Malaysia, Indonesia, Korea and the Philippines. In India, the crop is popular in the North-Eastern hills, the Eastern and Western Ghats, Punjab, Uttar Pradesh, Rajasthan and parts of Himachal Pradesh (Arora et al., 1980). It is grown for multiple uses, including green manure, green fodder and pulse, mainly as a *kharif* crop (sown in the rainy season and harvested in autumn). It has high yield potential and under good management practices yields of up to 28 q ha^{-1} have been recorded (Mukherjee et al., 1980). However, due to poor soil fertility and the mismanagement of resources, particularly the imbalanced use of fertilizers, much lower yields are generally achieved. Nitrogen and phosphorus fertilization are of prime importance for legumes and their deficiency appears to be the principal cause of lower yields (Bhati et al., 1988). The application of nitrogen along with adequate amounts of phosphorus improves growth, nodulation and grain yield (Tomar et al., 1984). Nitrogen application is known to increase vegetative growth, while phosphorus favours root development and reproductive growth. Niklyaev (1975) observed that adequate mineral fertilizer increased the seed yield and protein content from 1.4 to 2.0 times in the case of pulses. Hence, there is a need to develop an appropriate production technology for rice bean, particularly in relation to fertilizer management. Since very little information is available on the effect of nitrogen, phosphorus and potassium fertilization in rice bean, the present study was conducted to observe the effect of NPK enrichment on the growth, yield and quality of this crop.

Materials and methods

A field experiment was conducted in the Research Farm of CSK Himachal Pradesh Agricultural University, Palampur, having a clay loam soil with pH 6.5–7.0, during the *kharif* season of 2009 under rain-fed conditions. The average rainfall was 773.7 mm. The experiment was laid out in a random block design involving ten rice bean genotypes: JCR-20(S), IC-019352, LRB-168, LRB-40-2, LRB-164, IC-140796, JCR-152, IC-137195, IC-016789, JCR-20(D) and one check variety, BRS-2. The fertilizer treatments consisted of three NPK levels: 0:0:0 (T_0) control, 10:30:10 kg/ha (T_1) and 20:60:20 kg/ha (T_2). Each treatment was replicated thrice. The experimental plot measured 1.5 m × 1.0 m with plant and row spacing of 10 cm and 50 cm, respectively. Observations on flowering, maturity, total pods picked at maturity and seed yield were recorded on a plot basis, while the characters plant height, number of pod clusters/plant, number of pods/cluster, total number of pods/plant, pod length (cm) and number of seeds/pod were recorded for five randomly selected competitive plants from each plot. The crude protein was estimated using standard (AOAC, 1970) methods. The limiting amino acids tryptophan and methionine were estimated using the methods reported by Mertz et al. (1975) and Horn et al. (1946), respectively. The data were analysed as recommended by Panse and Sukhatme (1984).

Results

A) Morpho-physiological and yield traits

Plant height (cm)

Plant height 115 days after sowing varied from 216.51 (BRS-2) cm to 246.75 cm (JCR-20) and was significantly higher than in the check for all genotypes except LRB-168, JCR-152 and IC-137195 (Table 1). The fertilizer treatments also significantly affected the plant height, the tallest plants being observed in the T_1 treatment (245.00 cm), followed by T_2 (242.01 cm) and T_0 (229.32 cm). The interaction between fertilizers and genotypes was insignificant.

Days to maturity

Significant variation was observed between the rice bean genotypes for days to maturity and all the genotypes were significantly later than the check (Table 2). Non-significant variations were caused by the fertilizer treatments and by the genotype × fertilizer level interaction.

Table 1
Plant height (cm) of rice bean genotypes 115 days after sowing at different fertilizer levels

Fertilizer levels	Genotypes											
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	Mean
T_1^+	254.06	244.33	243.93	264.20	256.33	254.00	221.00	227.53	244.46	263.86	221.26	245.00
T_2^{++}	244.13	240.60	237.46	251.33	238.26	264.26	220.86	247.86	240.20	246.20	230.93	242.01
T_0^{+++}	242.06	241.73	210.13	228.66	238.00	251.26	226.80	226.20	237.13	223.20	197.33	229.32
G.M	246.75	242.22	230.51	248.06	244.20	256.51	222.88	233.86	240.60	244.42	256.51	
Parameters												
Factors	CD	CV										
Fertilizers	9.77	8.32										
Genotypes	18.72											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

Table 2
Days to maturity of rice bean genotypes at different fertilizer levels

Fertilizer levels	Genotypes											
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	Mean
T_1^+	131.66	124.00	124.66	128.00	127.00	130.00	130.00	132.33	124.66	129.66	116.33	127.12
T_2^{++}	132.66	129.00	129.66	124.66	128.66	130.66	130.33	134.33	129.33	128.00	118.33	128.69
T_0^{+++}	131.00	127.67	126.33	134.33	128.66	132.33	129.00	130.00	126.00	128.33	118.33	128.36
Mean	131.77	126.88	126.88	129.00	128.11	131.00	129.77	132.22	126.66	128.66	117.66	
Parameters												
Factors	CD	CV										
Fertilizers	NS	2.94										
Genotypes	2.36											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

Number of pods/plant

The mean values for pods per plant for the individual genotypes varied from 120.40 to 140.91 with the highest values for IC-140796 (140.91), JCR-20 (140.73) and JCR-152 (138.17) and the lowest values for the check variety, BRS-2 (120.40) and genotypes IC-016789 (125.35) and IC-135195 (129.64) (Table 3). The maximum number of pods was recorded in the T_2 treatment (138.56), followed by T_1 (132.23) and T_0 (126.29). The interaction between fertilizers and genotypes was insignificant.

Number of seeds/pod

The mean number of seeds/pod varied from 7.88 in IC-016789 to 8.76 in LRB-164 (Table 4). In general, longer pods had a higher number of seeds/pod and vice versa. All the genotypes were observed to be statistically at par with each other. Higher numbers of seeds/pod were recorded in LRB-164 (8.76), LRB-168 (8.62) and IC-019352 (8.48), and lower numbers in IC-016789 (7.88), IC-140796 (7.89) and JCR-152 (8.03). The fertilizer treatments affected the number of seeds/pod significantly, with values of 8.46 in T_2 and 8.50 in T_1 compared with 7.99 T_0.

Table 3
Number of pods/plant of rice bean genotypes at different fertilizer levels

Fertilizer levels	Genotypes											
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	Mean
T_1^+	138.53	133.26	128.46	133.46	127.13	128.60	141.60	133.53	125.33	140.20	124.46	132.23
T_2^{++}	145.60	140.26	129.60	131.20	141.80	154.93	142.33	128.46	128.93	146.53	134.33	138.56
T_0^{+++}	138.06	119.60	134.80	125.46	120.53	139.20	130.60	126.93	121.80	130.06	102.20	126.29
Mean	140.73	131.04	130.95	130.04	129.82	140.91	138.17	129.64	125.35	138.93	120.40	
Parameters												
Factors	CD	CV										
Fertilizers	NS	15.48										
Genotypes	NS											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

Table 4
Number of seeds/pod of rice bean genotypes at different fertilizer levels

Fertilizer levels	Genotypes											
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	Mean
T_1^+	8.58	8.86	9.90	8.13	8.66	8.22	7.92	8.30	8.04	8.63	8.22	8.50
T_2^{++}	8.32	8.79	8.60	8.74	8.76	8.16	8.37	8.56	7.50	8.53	8.72	8.46
T_0^{+++}	8.33	7.80	7.36	8.42	8.88	7.29	7.80	8.10	8.09	8.18	7.65	7.99
Mean	8.41	8.48	8.62	8.43	8.76	7.89	8.03	8.32	7.88	8.45	8.19	
Parameters												
Factors	CD	CV										
Fertilizers	0.40	9.78										
Genotypes	NS											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

Seed yield (q/ha)

Significant variation was observed between the rice bean genotypes for seed yield (q/ha) on a plot basis (Table 5), with values ranging from 18.53 q in LRB-164 to 24.84 q in JCR-20(S). In addition to JCR-20(S), genotypes IC-140796 (23.13 q/ha), IC-019352 (23.13 q/ha), and JCR-152 (23.07 q/ha) also had higher seed yield than the check BRS-2 (22.02 q/ha), but the difference was only significant for JCR-20(S). All the other genotypes were statistically at par with the check variety, except LRB-164 (18.53 q/ha) and LRB-40-2 (19.63 q/ha), which had significantly lower yields. Significant variation was observed between the fertilizer treatments, with the maximum seed yield in the T_2 treatment (24.27 q/ha), followed by T_1 (20.86 q/ha) and T_0 (20.09 q/ha). The T_2 treatment was significantly superior to both T_1 and the control, whereas T_1 and T_0 were statistically at par with each other.

B) Quality traits

Crude protein (%)

No significant variation was observed for crude protein (%) due to genotypes, fertilizers or the fertilizer × genotype interaction (Table 6). The mean value for crude protein varied from 24.98% in JCR-20(S) to 25.66% in JCR-152. All the genotypes except JCR-20(D) contained more crude protein than the check (24.01%).

Tryptophan (g/16 g N)

The variation in the tryptophan content (g/16 g N) due to fertilizers and the fertilizer × genotype interaction was non-significant, with mean values ranging from 1.08 g/16 g N in JCR-152 to 1.46 g/16 g N in LRB-40-2. All the genotypes except LRB-40-2 were statistically at par with the check (1.21 g/16 g N) (Table 7).

Table 5
Seed yield (q/ha) of rice bean genotypes at different fertilizer levels

Fertilizer levels	Genotypes											
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	Mean
T_1^+	22.90	24.73	19.70	20.66	17.33	23.04	20.13	21.86	18.85	17.81	22.46	20.86
T_2^{++}	31.41	24.47	21.83	22.62	19.94	25.88	29.18	24.74	27.44	19.98	19.91	24.27
T_0^{+++}	20.21	20.20	23.67	15.60	18.78	21.43	19.90	20.50	19.77	22.83	18.13	20.09
Mean	24.84	23.13	21.73	19.63	18.53	23.45	23.07	22.37	22.02	20.20	20.16	
Parameters												
Factors	CD	CV										
Fertilizers	2.34	21.80										
Genotypes	NS											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

Methionine (g/16 g N)

No significant variation was observed for methionine (g/16 g N) due to genotypes, fertilizers or the fertilizer × genotype interaction (Table 8). Only JCR-20(D) (5.50 g/16 g N) and IC-016789 (5.29 g/16 g N) contained more methionine than BRS-2 (5.22 g/16 g N).

Table 6
Crude protein content (%) of rice bean genotypes at different fertilizer levels

Fertilizer levels	Genotypes											Mean
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	
T_1^+	24.49	24.79	25.08	24.20	23.91	24.79	25.95	24.79	23.91	21.58	23.33	24.25
T_2^{++}	25.08	24.79	24.79	24.20	23.91	25.08	25.95	24.49	24.20	22.74	22.49	24.49
T_0^{+++}	25.37	24.79	24.20	23.91	24.49	24.79	25.08	24.20	24.20	23.91	24.20	24.47
Mean	24.98	24.79	24.59	24.10	24.10	24.88	25.66	24.49	24.10	22.74	24.01	
Parameters												
Factors	CD	CV										
Fertilizers	NS	7.01										
Genotypes	NS											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

Table 7
Tryptophan content (g/16 g N) of rice bean genotypes at different fertilizer levels

Fertilizer levels	Genotypes											Mean
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	
T_1^+	1.28	1.32	1.17	1.43	1.29	1.18	1.05	1.10	1.21	1.19	1.18	1.22
T_2^{++}	1.16	1.27	1.18	1.42	1.27	1.19	1.08	1.13	1.15	1.16	1.25	1.21
T_0^{+++}	1.22	1.30	1.25	1.52	1.23	1.15	1.10	1.12	1.13	1.11	1.20	1.21
Mean	1.22	1.30	1.20	1.46	1.26	1.17	1.08	1.12	1.16	1.15	1.21	
Parameters												
Factors	CD	CV										
Fertilizers	NS	12.16										
Genotypes	0.14											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

Discussion

The fertilizer treatments significantly affected the plant height, seeds/pod and seed yield. Iqbal et al. (1998) also reported that the application of 50 kg ha^{-1} nitrogen and 75 kg ha^{-1} phosphorus resulted in a significant increase in the height of rice bean plants over the control. Seed yield and its contributing traits, except number of seeds/pod, increased linearly with a rise in the dose of fertilizer, whereas plant height and number of seeds/pod were maximum in the T_1 treatment, followed by T_2 and the control. Other parameters (days to 25%

Table 8
Methionine content (g/16 g N) of rice bean genotypes at different fertilizer levels

Fertilizer levels	Genotypes											Mean
	JCR-20(S)	IC-019352	LRB-168	LRB-40-2	LRB-164	IC-140796	JCR-152	IC-137195	IC-016789	JCR-20(D)	BRS-2	
T_1^+	4.29	5.01	4.78	4.79	5.05	4.68	4.38	4.78	5.13	6.20	5.97	5.00
T_2^{++}	4.19	5.00	4.83	5.03	4.91	4.72	4.72	4.78	5.07	5.96	5.52	4.97
T_0^{+++}	4.48	5.09	5.39	5.26	5.49	5.46	4.25	4.37	5.67	4.36	4.16	4.91
Mean	4.32	5.03	5.00	5.03	5.15	4.95	4.45	4.64	5.29	5.50	5.22	
Parameters												
Factors	CD	CV										
Fertilizers	NS	17.00										
Genotypes	NS											
Fert.×Geno.	NS											

$^+$N: P: K = 10:30:10; $^{++}$N: P: K = 20:60:20; $^{+++}$ N: P: K = 0:0:0; NS = non-significant

flowering, days to 50% flowering, days to maturity, number of pods/cluster, number of pods/plant and pod length) were not affected significantly by the fertilizer treatments. The interaction between genotypes and fertilizer levels was found to be insignificant for all the parameters studied. Significant variation was also observed between the rice bean genotypes for plant height 115 days after sowing and days to maturity. Genotypes JCR-20, IC-140796, IC-019352 and JCR-152 had higher seed yields than the check, but the difference was only significant for JCR-20. Mohapatra et al. (1996) reported that the seed yield of rice bean genotypes responded positively and significantly to N fertilization up to 40 kg ha^{-1} and to P fertilization up to 60 kg P ha^{-1}, whereas Khanda and Mishra (1998) recorded maximum seed yield in rice bean when N was applied at 40 kg ha^{-1}, followed by 60 kg ha^{-1}. Vikrant et al. (2006) also recorded maximum seed yield in cowpea genotypes, when P was applied at 60 kg ha^{-1}. The results of the present research corroborate the previous findings. Quality traits such as crude protein (%), tryptophan (g/16 g N) and methionine (g/16 g N) were not significantly affected by the fertilizer treatments. Bishop et al. (1976) also observed that NPK application had a non-significant effect on the protein content of bean seeds. Thus, the present study revealed that fertilization has a crucial role in crop productivity and that an NPK ratio of 20:60:20 kg ha^{-1} is promising for enhancing the rice bean yield without any effect on the seed protein and amino acid profiles.

References

AOAC (1970): *Official Methods of Analysis of the Association of Official Analytical Chemists.* 11th edition. Washington, D.C.

Arora, R. K., Chandel, K. P. S., Joshi, B. S. (1980): Rice bean: tribal pulse of eastern India. *Econ. Bot.*, **34**, 260–263.

Bhati, D., S., Mathur, J. R., Sharma, R. C. (1988): Response of moth bean [*Vigna aconitifolia* (Jacq.) Marechal] to graded levels of N and P. *Ann. Arid Zone,* **27**, 63–64.

Bishop, R. F., Smeltzer, G. G., MacEachern, C. (1976): Effect of nitrogen, phosphorus and potassium on yield, protein content and nutrient levels in soybeans, field beans and faba beans. *Commun. Soil Sci. Plant Anal.,* **7**, 387–404.

Horn, M. J., Jones, D. D., Blum, A. E. (1946): Colorimetric determination of methionine in proteins and foods. *J. Biol. Chem.,* **116**, 313–320.

Iqbal, K., Tanveer, A., Tahir, M. (1998): Growth and yield response of rice bean (*Vigna umbellata*) fodder to different levels of N and P. *Pak. J. Biol. Sci.,* **1**, 212–214.

Khanda, C. M., Mishra, P. K. (1998): Effect of plant density and nitrogen fertilization on growth and yield of rice bean. *Indian J. Agron.,* **43**, 700–703.

Mertz, E. T., Jambunathan, R., Mishra, P. S. (1975): *Protein Quality. Agricultural Research Bull. No. 7*, Purdue University, USA. p. 9.

Mohapatra, A. K., Piakray, R. K., Haldar, J. (1996): Response of rice bean (*Vigna umbellata*) to nitrogen. *Ann. Agri. Res.*, **17**, 445–446.

Mukherjee, A. K., Roquib, M. A., Chatterjee, B. N. (1980): Rice bean for the scarcity period. *Indian Farm.,* **30**, 26–28.

Niklyaev, V. S. (1975): The effect of seed rate and fertilizers on the growth, development and yield of pulses. *Vestnik Selskohoziaistvenno*, **11**, 29–35.

Panse, V. G., Sukhatme, P. V. (1984): *Statistical Methods for Agricultural Workers*. Indian Council of Agricultural Research, New Delhi. p. 381.

Singh, S. P., Mishra, B. K., Pant, K. C. (1980): Major food constituents of rice bean (*Vigna umbellata*). *J. Food Sci. Technol.,* **17**, 238–240.

Tomar, S. S., Sharma, R. K., Namdeo, K. N. (1984): Performance of urd bean varieties under various row spacing and fertility levels. *Legume Res.,* **17**, 51–53.

Vikrant, H. S., Malik, C. V. S., Singh, B. P. (2006): Response of cowpea to farm yard manure and phosphorus fertilization. *Crop Res.,* **31**, 222–225.

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GENOTYPE × ENVIRONMENT INTERACTION AND STABILITY ANALYSIS ON BARLEY (*Hordeum vulgare* L.) LINES IN ALGERIA

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The objectives of this research were to assess the genotype × environment interaction and to identify barley (*Hordeum vulgare* L.) genotypes with high stability for grain yield, yield components, straw height, ear length, heading time, straw yield and harvest index. Eighteen barley breeding lines and one commercial cultivar were evaluated under field conditions during five growing seasons. The trials were carried out under both rainfed and irrigated conditions during the first four years and under rainfed conditions only during the last growing season. Stability studies showed significant differences between the environments for all the traits and between the genotypes for thousand-grain weight, heading time and ear length. The genotype × environment interaction was, however, not significant according to the Finlay–Wilkinson analysis. The analysis of correlations between the various traits showed the importance of selecting for earliness, high number of grains/ear, stem height and ear length in order to obtain acceptable grain yields under drought-stressed conditions.

Key words: genotypes, environment, interaction, barley, stability, yield components

Introduction

In Algeria, where the environmental conditions are not always optimum (because of irregular quantity and distribution of rainfall during the cereal growing cycle, tillage and fertilization), the phenotypic performance of a genotype differs greatly under diverse agroclimatic conditions. This variation is due to the genotype × environment interaction, which reduces the stability of a genotype under different environmental conditions (Ali et al., 2003). The genotype × environment interaction becomes particularly important when the rank of breeding lines changes across environments. This change in ranking has been defined as a cross-over genotype × environment interaction (Baker, 1988). The genotype × environment interaction prevents the extrapolation of the results of agronomic evaluations from one location to another, thus requiring expensive

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trials at multiple locations (Avis et al., 2009). Stability studies in cereals (barley and wheat) are an essential part of breeding programmes prior to the release of a new variety, and a high number of locations and years are usually necessary for the adequate evaluation of cultivars. Many models have been developed to measure the stability of various parameters and estimate the part of the variation that is due to the genotype × environment interaction. Becker and Leon (1988) stated that all stability procedures based on quantifying genotype × environment interaction effects follow the dynamic concept. These include (i) the procedures for partitioning genotype × environment interactions according to Wricke's (1962) ecovalence and Shukla's (1972) stability of variance, (ii) those using a regression approach such as that proposed by Finlay and Wilkinson (1963), Eberhart and Russell (1966) and Perkins and Jinks (1968), and (iii) non-parametric stability analyses. A genotype is considered to have good dynamic stability if it has a high mean yield with a low degree of fluctuation in yielding ability when grown under diverse climatic conditions.

The current investigation was carried out to determine the potential of promising barley genotypes in terms of the stability of grain yield, yield components, heading and morphological traits under the environmental conditions of the National Advanced School of Agronomy (ENSA) of Algiers (Algeria) during five years of field experiments.

Materials and methods

The experimental material consisted of nineteen barley (*Hordeum vulgare* L.) genotypes, one commercial cultivar (Rihane), fourteen doubled haploid lines and four advanced lines (Table 1). The doubled haploid lines (DH) were obtained using the *H. bulbosum* method (Kasha and Kao, 1970; Adamski, 1979) from F_1 hybrids of three combinations (Motan × California Mariout 67, Saïda × Ensenada and Prato × California Mariout 67) and the advanced lines were obtained using genealogical selection from three crosses (Saïda × Jaidor, Jaidor × Ensenada and Saïda × Apizaco) by Hanifi (1999) (Table 1). These genotypes were analysed in field experiments laid out in a randomized complete block design with three replications. The experiments were performed under rainfed conditions and with additional irrigation in the 1996–1997, 1997–1998, 1998–1999 and 2007–2008 growing seasons and under rainfed conditions in 2002–2003 (Table 2) at the National Advanced School of Agronomy of Algiers. Each genotype was sown in 3.6 m^2 plots (1.2 m × 3 m) consisting of 6 rows with a 20 cm row space, at a density of 300 seeds/m^2. The plots were fertilized with 30 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1} at planting and 30 kg N ha^{-1} in spring at stem elongation in all the tests. Details of the experimental locations (rainfed conditions and with additional irrigation), year and planting date of the various trials are given in Table 2.

The variables analysed were number of tillers/m^2 ($T \cdot m^{-2}$, counted at full tillering), number of ears/m^2 ($E \cdot m^{-2}$), number of grains/ear (Nbrgr), thousand-grain weight (GW), grain yield (GrY), straw yield (StrY), harvest index (H.I.), stem height (StH), ear length (EarL) and heading time (Head). All the measurements were carried out at harvest on all the plants of the central 1 m^2 of each plot except heading time, which was assessed when 50% of the ears had emerged. The stem height was measured from the surface of the ground to the base of the ear, while the ear length was measured from the base to the top of the ear (awns were not included).

Table 1
Crossings, DH line numbers and genealogical lines

Crossings and codes	Code of DH lines obtained from F_1	Genealogical lines
Motan × California Mariout 67	29, 130, 48, 277, 3, 167, 226, 202 and 196	–
Saïda × Ensenada (18/17)	18/17/7 and 18/17/2	–
Saïda × Jaidor (18/3)	–	18/3/2b
Prato × California Mariout 67 (15/14)	15/14/10, 15/14/14 and 15/14/19	–
Jaidor × Ensenada (3/17)	–	3/17/2a and 3/17/2b
Saïda × Apizaco	–	18/16/1/2

Table 2
Details of experimental locations (rainfed and irrigated), year and planting date

Locations	Growing seasons	Rainfall (mm)		Irrigation (mm)	Stages of irrigation and total water (rain + irrigation) from sowing to harvest	Date	
		Annual rainfall	From sowing to harvest			Sowing	Harvest
Rainfed	1995–1996	742.80	651.9			18/12/95	13/06/96
	1996–1997	382.24	215.8			12/12/96	12/06/97
	1997–1998	722.40	372.9			11/01/98	13/06/98
	2002–2003	772.00	450.0			30/12/02	28/05/03
Additional irrigation	1995–1996	742.8	651.9	30.0	- flowering Total: 681.9	18/12/95	13/06/96
	1996–1997	382.24	215.8	60.0 60.0 60.0	- stem elongation - flowering - grain filling Total: 395.8	11/12/96	12/06/97
	1997–1998	722.40	372.9	60,0 40.0	- heading - grain filling Total: 472.9	13/01/98	13/06/98
	2007–2008	720.9	216.9	15.0 40.0 20.0 10.0	- tillering - stem elongation - flowering - grain filling Total: 301.9	08/01/08	

A combined analysis of variance was undertaken across environments using model 1, where the genotype and environment effects are random (Annicchiarico, 2002). The data were analysed as a three-factorial experiment using analysis of variance (ANOVA). The factors were the genotype (*G*) and the environment (*L*) crossed with the factor block (*B*). The observed yield response *Rijr* of genotype *i* in the location or environment *j* and block *r* is:

$$Rijr = \mu + G_i + L_j + B_r\,(L_j) + GL_{ij} + e_{ijkr},$$

where μ is the general mean.

The analysis of variance for the adaptation study of Finlay and Wilkinson (1963) was carried out using the "GEST" program reported by Ukai (2000), based on the model of Eberhart and Russell (1966):

$$Y_{ij} = \mu_i + \beta_{ilj} + \sigma^2_{ij}$$

where Y_{ij} is the mean for the i^{th} genotype in the j^{th} environment, μ_i is the general mean, β_i is the regression coefficient of the i^{th} genotype on l_j (environmental index), which is defined as the mean deviation for all genotypes at a given location from the overall mean, and σ^2_{ij} is the deviation from regression for the i^{th} genotype at the j^{th} location.

$$\beta_i = ((\Sigma Y_{ij}\, l_j))/(l_j^2),\ s_{di}^2 = [\Sigma \sigma_{ij}^2/(l-2)] - S_e^2/r$$

where r = replication and S_e^2 = estimated for pooled error.

Studying the variance components of the genotype × environment interaction allowed the stability of the genotypes to be determined (Eberhart and Russell, 1966). Eberhart and Russell (1966) used joint linear regression, where the studied trait of each genotype, for example the grain yield, is regressed on the environmental mean grain yield. The performance of a genotype is generally broken up into environment (linear), genotype × environment (linear) and deviations of the model of regression. Each genotype is generally characterized by its mean, its regression coefficient (β_i) and its deviation (s^2_{di}) from regression. According to Eberhart and Russell (1966), a value of regression equal to one ($\beta_i = 1$) coupled with minimum deviation ($s^2_{di} = 0$) indicates average stability. A regression coefficient above 1.0 describes genotypes with higher sensitivity to environmental change and greater specificity of adaptability to high-yielding environments. A regression coefficient below 1.0 indicates specific adaptation to low-yielding environments (Wachira et al., 2002). Linear regression analysis between the environmental index and the mean value of each genotype for the various traits was carried out when the genotype × environment interaction was significant. The broad sense heritability was calculated according to the following formula (Gallais, 1990):

$$H^2 = \frac{\sigma_g^2}{\sigma_{ph}^2} = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gl}^2}{l} + \frac{\sigma_e^2}{rl}},$$

where σ_g^2 = genotypic variance, σ_{gl}^2 = genotype × environment interaction variance, σ_e^2 = environmental variance, and g, ph, l and r are, respectively, genotype, phenotype, environment and block number or replications.

Results and discussion

Analysis of variance revealed a significant environment effect ($P<0.001$) for all the traits studied (Table 3). This effect was mainly due to the quantity of rain, which varied from one year to another, ranging from 215.8 mm (1997) to 651.9 mm (1996) (Table 2). Additional irrigation improved the number of grains/ear, the grain yield and the ear length because irrigation was started at the stem elongation stage (Table 2).

The genotype effect was significant at $P<0.05$ for number of ears per plant, number of grains per ear and grain yield, and at $P<0.001$ for number of tillers/m², thousand-grain weight, heading date and ear length. The significance of the factor 'Genotype' suggested that genetic differences existed among the genotypes for these traits. The environment × genotype interaction was significant at $P<0.01$ for number of tillers/m², grain yield and straw yield, and at $P<0.001$ for thousand-grain weight, straw height and ear length, indicating significant changes in the ranking order of the genotypes for these traits from one year to another.

Table 3
Mean squares and degree of freedom from analysis of variance and explained variance (%) for the traits studied

Traits	Environment		Genotype		Environment × genotype		Error (df = 324)
	M.S. (df = 8)	%	M.S. (df = 18)	%	M.S. (df = 144)	%	
$T \cdot m^{-2}$	2961020.94***	57.56	92269.88***	4.04	33955.88**	11.88	22688.14
$E \cdot m^{-2}$	1190799.73***	49.01	38297.72*	3.55	21052.66 ns	15.59	16559.80
Nbrgr	1986.68***	42.17	99.62*	4.76	47.79 ns	18.26	38.30
GW	1138.98***	38.13	203.03***	15.29	44.04***	26.54	13.86
GrY	904146.38***	47.55	39146.01*	4.63	19096.39**	18.07	11725.0
StrY	1036107.69***	13.81	163728.33 ns	4.91	127536.15**	30.59	83429.75
H.I.	0.267***	32.03	0.014 ns	3.89	0.009 ns	20.47	0.008
Head	2138.74***	61.54	97.58***	6.32	33.68**	17.44	11.70
Height	6313.80***	62.52	89.06 ns	1.98	88.37***	15.75	41.02
EarL	5.70***	6.76	9.16***	23.11	1.31***	27.58	0.85

NB: Genotype and environment effects were tested compared to the interaction variance of ANOVA. $T \cdot m^{-2}$ = number of tillers/m², $E \cdot m^{-2}$ = number of ears/m², Nbrgr = number of grains/ear, GW = thousand-grain weight, GrY = grain yield, StrY = straw yield, H.I. = harvest index, Head = heading time, EarL = ear length, % = explained (%). *, **, *** = significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively; ns = non-significant

The barley traits were most markedly affected by the environment (6.76% to 62.52%), except for ear length, where the genotype effect was higher than the environment effect. The genotype effect (1.98% to 23.11%) was lower than the genotype × environment interaction (11.88% to 27.58%) in each case (Table 3).

The residual coefficients of variation (Table 4) were low for heading time (3.47%), average for thousand-grain weight (9.36%), ear length (12.53%) and number of grains/ear (13.20%), and very high for number of ears/m² (30.38 %), grain yield (25.62%) and number of tillers/m² (22.62%). The genetic coefficients of variation (Table 4) revealed great genetic variation between the genotypes for ear length (7.65%), number of tillers/m² (6.98%), thousand-grain weight (6.38%) and grain yield (6.42%) and low variation for number of grains/ear (2.95%) and heading time (1.56%).

Table 4
Variance components, broad sense heritability (H^2), variation coefficients and general means of traits having significant genotypic differences

	GW	Nbrgr	GrY	$T \cdot m^{-2}$	EarL	Head	$E \cdot m^{-2}$
Sg^2	5.889	1.920	742.578	2159.778	0.291	2.367	638.743
Sgl^2	10.060	3.162	2423.688	3755.917	0.177	7.326	1497.284
Se^2/r	4.620	12.768	3941.776	7562.712	0.260	3.900	5519.935
H^2	0.286	0.108	0.104	0.160	0.400	0.174	0.083
Error CV (%)	9.36	13.20	25.62	22.61	12.53	3.47	30.38
Genetic CV (%)	6.10	2.95	6.42	6.98	7.65	1.56	5.97
Range	33.65–44.67 g	44.22–50.27	349.07–488.46 g	581.91–785.27	6.01–8.17 cm	97.00–103.33	366.72–509.37
General means	39.77 g	46.90	424.43 g	666.19	7.05 cm	98.64	426.56

GW = thousand-grain weight, Nbrgr = number of grains/ear, GrY = grain yield, $T \cdot m^{-2}$ = number of tillers/m², Head = heading time, EarL = ear length, $E \cdot m^{-2}$ = number of ears/m².

The broad sense heritability calculation (Table 4) revealed high heritability for ear length (0.400), while thousand-grain weight (0.286), heading time (0.174), number of tillers/m² (0.160), number of grains/ear (0.108) and grain yield (0.104) had low heritability.

Mean values (Table 4) for the 19 genotypes over all environments ranged from 379.82 g to 488.46 g/m² for grain yield, 33.64 to 44.67 g for thousand-grain weight, 44.22 to 50.59 for number of grains/ear, 581.95 to 785.27 for number of tillers/m², 6.01 to 8.17 cm for ear length and 95.67 to 103.33 days for heading time. Genotypes Rihane, 3, 18/3/2b, 277, 18/16/1/2, 48, 18/17/2, 226 and 15/14/10 gave the highest grain yield (Table 5). The highest number of tillers/m² was observed for genotypes 277, 167, 130, 3, Rihane, 18/17/2 and 18/16/1/2, whereas genotypes 18/17/7 (509.37), 48 (475.20), 277 (456.59), 18/17/2 (455.58), 18/17/7 (453.30) and 18/16/1/2 (449.17) had the highest number of ears/m². Genotypes 15/14/19, 15/14/14 and 3/17/1 had the highest number of grains/ear (Table 5), whereas genotypes 18/17/7 (44.67 g), 3/17/1/2 (43.34 g), 226 (42.22 g), 3 (42.24 g) and Rihane (41.70 g) had the highest thousand-grain weight. The highest ear length was noted for genotypes 18/3/2b (8 cm) and 3, 196, 18/17/7 (approximately 7.5 cm). With regard to heading, the earliest genotypes were 3 (95.67 days), 48 (97.26 days), 15/14/19, 15/14/14, 18/17/7, Rihane and 29 (all approximately 97 days). The latest were 167 (103.33 days), 130 (102.11 days), 18/3/2 b (100.44 days) and 202 (100.22 days).

Table 5
Genotype means (M) and coefficients of determination (R^2ij) of various traits

Genotypes	GrY		$T \cdot m^{-2}$		Nbrgr		EarL		Head		$E \cdot m^{-2}$		GW	
	M (g)	R^2ij	M	R^2ij	M	R^2ij	M(cm)	R^2ij	M(days)	R^2ij	M	R^2ij	M (g)	R^2ij
Rihane	488.46	0.81	693.4	0.97	46.30	0.33	6.01	0.06	97.30	0.95	439.55	0.65	41.70	0.83
29	405.21	0.35	643.2	0.77	46.90	0.81	6.71	0.36	97.00	0.72	395.85	0.71	37.64	0.49
18/17/7	407.77	0.70	646.44	0.93	46.66	0.74	7.47	0.45	97.52	0.90	509.37	0.31	44.67	0.16
3	479.24	0.83	723.32	0.97	45.38	0.72	7.50	0.25	95.67	0.47	453.30	0.94	42.21	0.87
167	408.32	0.76	772.74	0.93	44.22	0.72	6.49	0.32	103.33	0.90	378.43	0.73	40.04	0.57
277	477.19	0.74	785.27	0.95	47.86	0.69	6.01	0.03	98.74	0.19	456.59	0.78	33.65	0.96
3/17/1	379.83	0.60	642.21	0.53	49.86	0.82	8.17	0.40	99.26	0.01	366.72	0.92	40.57	0.86
18/16/2	450.80	0.50	665.65	0.88	44.59	0.92	7.29	0.48	98.63	0.92	449.17	0.86	40.11	0.20
202	403.49	0.71	608.86	0.76	44.48	0.49	7.17	0.07	100.22	0.11	388.38	0.58	40.97	0.76
15/14/10	421.06	0.65	599.13	0.87	47.28	0.79	7.28	0.23	98.37	0.85	425.36	0.95	38.05	0.85
15/14/14	416.36	0.77	645.65	0.97	50.27	0.55	6.67	0.08	97.89	0.94	395.55	0.94	36.70	0.53
18/3/2b	479.79	0.50	656.22	0.95	47.74	0.79	8.00	0.21	100.44	0.95	427.09	0.89	40.25	0.77
3/17/1/2	349.07	0.70	626.73	0.83	46.01	0.74	7.10	0.30	99.22	0.06	378.12	0.86	43.34	0.70
226	424.22	0.75	636.28	0.89	47.46	0.75	7.28	0.50	98.04	0.74	410.33	0.98	42.22	0.84
18/17/2	437.61	0.67	694.40	0.94	46.04	0.81	7.17	0.00	96.26	0.89	455.58	0.88	42.08	0.60
196	416.66	0.63	606.30	0.92	48.28	0.47	7.49	0.46	99.19	0.87	398.77	0.80	37.41	0.89
130	379.82	0.64	756.73	0.93	46.74	0.57	6.61	0.03	102.11	0.76	440.31	0.96	36.04	0.28
15/14/19	396.31	0.36	581.95	0.73	50.59	0.91	6.57	0.11	97.63	0.57	404.01	0.72	39.09	0.67
48	443.03	0.63	673.12	0.93	44.45	0.92	6.94	0.66	97.26	0.78	475.20	0.91	38.95	0.85
ppds	58.09		80.47		3.31		0.41		1.83		68.75		1.99	

R^2ij = coefficient of determination between environment index and genotypic mean; Environment index = mean of all genotypes; GW = thousand-grain weight, Nbrgr = number grains/ear, GrY = grain yield, $T \cdot m^{-2}$ = number of tillers/m², $E \cdot m^{-2}$ = number of ears/m², Head = heading time, EarL = ear length

Depending on the genotype, high grain yield was the result of various combinations of yield components. The nine best genotypes for grain yield could be classified in three categories for heading: early genotypes ($\approx$97 days), like Rihane, 18/17/2, 3 and 48; genotypes with intermediate precocity ($\approx$99 days), like 18/16/1/2, 277, 196, 15/14/10 and 226; and late genotypes ($\approx$102 days) like 3/18/2b. Early genotypes are able to escape a moisture deficit at the end of the growing cycle and can be cultivated in areas where this stress has a strong probability of occurrence (Algerian high plains). Genotypes with average precocity can be cultivated in zones where moisture stress is less likely to occur (littoral plains). Late genotypes can be cultivated in zones with additional irrigation (Saharan zones).

The correlations between traits showed that grain yield was mainly correlated with straw yield ($P\leq0.001$), number of grains/ear ($P\leq0.001$) and stem height ($P\leq0.01$) under rainfed conditions and with number of tillers/m^2 ($P\leq0.001$), harvest index ($P\leq0.001$) and straw yield ($P\leq0.01$) under irrigated (Table 6). The number of grains/ear was correlated positively with ear length ($P\leq0.01$ for irrigated and rainfed conditions) and straw height ($P\leq0.001$ and $P\leq0.01$ for irrigated and rainfed conditions, respectively). Number of ears/m^2 ($P\leq0.001$) and number of tillers/m^2 ($P\leq0.01$ and $P\leq0.001$ for irrigated and rainfed conditions, respectively) were correlated negatively with straw height. These correlations show the importance of the straw yield in determining the barley grain yield, and of ear length and straw height in determining the number of grains/ear and number of ears/m^2. These results corroborate those reported by Bouzerzour et al. (1996; 2000). Irrigation improved the grain yield through enhancing the number of tillers/m^2, the number of ears/m^2 and the harvest index ($P\leq0.001$).

Under rainfed conditions the positive associations between stem height and grain yield and thousand-grain weight and between thousand-grain weight and ear length suggest that the latter trait takes part in grain filling. These results confirm those reported by Bouzerzour and Monneveux (1993), Abbassène et al. (1998) and Bahlouli et al. (2001). According to Gate et al. (1992; 1993), Ben Abdallah and Bensalem (1993), Mekliche et al. (1993) and Djebrani (2000), high straw confers better tolerance to drought thanks to the carbon products it contains, which ensure grain filling in the case of final moisture deficit. According to Bagga et al. (1970) a genotype with high straw also has a deep root system, which confers important water uptake capacity.

The negative effect of lateness on thousand-grain weight and grain yield is due to moisture deficit during grain filling. Under drought-stressed conditions, it is consequently important to choose early genotypes that can finish their life cycle before moisture deficit occurs. According to Debaek et al. (1996), the grain yield of wheat is more closely related to ear fertility than to grain size. The present results show that the correlations between grain yield and stem height ($P\leq0.01$) and grain yield and number of grains/ear ($P\leq0.001$) are positive and significant only under rainfed conditions (Table 6). Late heading has a positive effect on the number of grains/ear ($P\leq0.001$) under irrigated conditions and a negative effect on the thousand-grain weight under rainfed conditions ($P\leq0.001$) (Table 6).

Table 6
Correlations between traits

Correlations	Treatments (N=76)		Correlations	Treatments (N=95)	
	Irrigated	Rainfed		Irrigated	Rainfed
GrY – StrY	0.30**	0.59***	Nbrgr – Head	0.54***	0.09
GrY – Height	–0.24*	0.35**	Nbrgr – H.I.	–0.38***	–0.36**
GrY – H.I.	0.43***	–0.14	Nbrgr – EarL	0.32**	0.37**
GrY – E·m^{-2}	0.34***	–0.29*	Nbrgr – E.m^{-2}	–0.34***	–0.12
GrY – T. m^{-2}	0.62***	0.22	Nbrgr – Height	0.40***	0.37**
GrY – Nbrgr	–0.20*	0.56***	E· m^{-2} – T·m^{-2}	0.45***	0.37**
GW – Height	–0.19	0.60***	E· m^{-2} – Heading	–0.55***	–0.52***
GW – Nbrgr	–0.54***	0.09	E· m^{-2} – StrY	0.37***	0.15
GW – H.I.	0.38***	0.14	T· m^{-2} – Height	–0.33**	–0.49***
GW – Head	–0.13	–0.51***	Nbrgr – StrY	–0.18	0.39***
GW – EarL	0.15	0.25*	E m^{-2} – Height	–0.52***	–0.51***
GW – T· m^{-2}	–0.08	–0.55***	EarL – Height	0.09	0.30**

T·m^{-2} = number of tillers/m^2, E·m^{-2} = number of ears/m^2, Nbrgr = number of grains/ear, GW = thousand-grain weight, GrY = grain yield, StrY = straw yield, H.I. = harvest index, Head = heading time, Height = stem height, EarL = ear length; *, ** and *** = significant at P≤0.05, P≤0.01 and P≤0.001, respectively

The combined analysis of stability (Table 7) showed highly significant differences (P<0.001) between the environments for number of tillers/m^2, thousand-grain weight, grain yield, straw yield, heading time and stem height and significant differences (P<0.05) for ear length. The environment effect is likely to be due to differences in the quantity of water received by the various trials (Tarakanovas and Ruzgas, 2006). Indeed, the correlations between water quantity on the one hand and number of grains/ear and grain yield on the other (Table 8) are, respectively, significant (P<0.05) to very highly significant (P<0.001) in 12 cases out of 19 ($r_{[grain\ number\ -\ water]}$ = 0.80* for genotype mean) and significant (P<0.05) to highly significant (P<0.01) in 4 cases out of 19 ($r_{[grain\ yield\ -\ quantity\ of\ water]}$ = 0.59 for genotype mean). The correlation coefficients between the quantity of water and thousand-grain weight were negative and non-significant except for one genotype (15/14/19; r = –0.75*). The increase in the number of grains due to additional irrigation led to a reduction in thousand-grain weight because of the competition between grains on the same ear.

The differences between genotypes were highly significant (P<0.001) for thousand-grain weight, heading time and ear length. The genotype × environment interaction was non-significant for all the traits (Table 7). The linear interaction was highly significant (P<0.01) for heading time only when tested against the pooled error, indicating differences between the regression coefficients.

Table 7
Mean squares and degrees of freedom for stability parameters in the Finlay–Wilkinson analysis (Hardwick and Wood, 1972) for the traits studied

Traits	Genotype (df = 18)	Environment (df = 8)	Interaction G × E (df = 144)	Interaction (G × E)	
				Heterogeneity (df = 18)	Residual (df = 126)
T· m^{-2}	31666.13 ns	1130841.06***	10814.13 ns	22281.79 ns	9175.89 ns
GW	70.65***	399.50***	14.49 ns	23.02 ns	13.27 ns
GrY	13048.66 ns	301382.13***	6365.46 ns	3524.41 ns	6771.33 ns
StrY	62702.29 ns	351616.07***	33235.17 ns	35172.52 ns	32958.40 ns
Head	32.53***	712.91***	11.23 ns	24.49**	9.33 ns
Height	30.90 ns	2094.76***	28.81 ns	19.75 ns	30.10 ns
EarL	3.05***	1.71*	0.42 ns	0.33 ns	0.43 ns

NB: Genotype and environment effects were tested compared to the residual variance of ANOVA (Table 5); T·m^{-2} = number of tillers/m^2, GW = thousand-grain weight, GrY = grain yield, StrY = straw yield, Head = heading time, Height = stem height, EarL = ear length; *, **, *** = significant at P≤0.05, P≤0.01 and P≤0.001, respectively; ns = non-significant

Table 8
Correlations between means of various traits and total water (rain + irrigation) for various genotypes (df = 7)

Genotypes	Traits						Corr. S
	GrY	Nbrgr	GW	EarL	Height	Head	
Genot. mean	0.59	0.80*	–0.49	0.57	0.69*	–0.02	2
Rihane	0.44	0.27	–0.38	0.19	0.59	–0.11	0
29	0.21	0.78*	0.41	0.25	0.68*	0.19	2
18/17/7	0.74*	0.55	–0.07	0.67*	0.72*	–0.01	3
3	0.53	0.49	0.26	–0.76*	0.15	–0.28	1
167	0.64	0.69*	–0.57	0.33	0.64	0.22	1
277	0.33	0.62	–0.39	0.03	0.55	–0.22	0
3/17/1	0.30	0.92***	–0.41	0.22	0.63	0.77*	2
18/16/2	0.41	0.81**	–0.31	0.24	0.71*	–0.18	2
202	0.68*	0.68*	0.01	0.09	0.47	–0.53	2
15/14/10	0.63	0.75*	–0.56	0.25	0.63	0.12	1
15/14/14	0.34	0.37	–0.62	0.23	0.32	–0.14	0
18/3/2b	0.34	0.86**	–0.51	0.37	0.65	–0.14	1
3/17/1/2	0.82**	0.69*	–0.57	0.41	0.88**	0.43	3
226	0.49	0.89**	–0.44	0.64	0.73*	0.15	2
18/17/2	0.26	0.70*	–0.21	0.29	0.62	–0.17	1
196	0.71*	0.30	–0.20	0.22	0.76*	0.15	2
130	0.08	0.39	–0.51	0.23	0.59	0.21	0
15/14/19	0.31	0.77*	–0.75*	0.01	0.74*	–0.44	3
48	0.37	0.76*	–0.55	0.37	0.71*	0.08	2
Total corr. S.	4	12	1	2	8	1	28

NB: Genotype and environment effects were tested compared to the residual variance of ANOVA (Table 5); Nbrgr = number of grains/ear, GW = thousand-grain weight, GrY = grain yield, Head = heading time, Height = stem height, EarL = ear length; Genot. Mean = mean of all genotypes, Corr. S = number of significant correlations; *, ** and *** = significant at P≤0.05, P≤0.01 and P≤0.001, respectively; Correlations for number of tillers/m^2 and number of ears/m^2 were not significant.

The coefficients of determination (R^2ij) (Table 5) between genotype means and the environmental index ranged from 0.73 to 0.97 for number of tillers/m^2, 0.33 to 0.92 for number of grains/ear, 0.06 to 0.95 for heading time, 0.36 to 0.81 for grain yield, 0.31 to 0.96 for number of ears/m^2, 0.16 to 0.96 for thousand-grain weight and 0.03 to 0.66 for ear length. These coefficients confirmed the effect of the environment on the traits of various genotypes. The coefficient of determination (R^2ij) can also be used to predict the performance of genotypes (Pinthus, 1973).

Conclusions

Stability analysis provides a general indication of the response patterns of genotypes to environmental changes. This has considerable importance in the choice of varieties to cultivate in various locations. In this study, the environment effect was higher for all traits than the genotype and genotype × environment interaction effects. This last effect was non-significant according to the Finlay–Wilkinson analysis, indicating that the genotypes studied had good stability. Genotypes Rihane, 18/3/2b, 3, 277, 18/16/1/2, 48, 18/17/2, 226 and 15/14/10 showed the highest grain yield. Among them, the genotypes Rihane, 18/17/2, 3 and 48 were early, genotypes 277, 18/16/1/2, 15/14/10 and 226 intermediate and genotype 18/3/2b late. Correlation analysis between the various traits pointed out the importance of number of grains/ear, stem height, ear length and earliness for obtaining an acceptable grain yield under drought-stressed conditions. Irrigation improved the grain yield mainly by enhancing the number of tillers/m^2, number of ears/m^2 and harvest index. The broad sense heritability of ear length and thousand-grain weight makes it possible to select effectively for these traits.

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References

Abbassène, F., Bouzerzour, H., Hachemi, L. (1998): Taux et durée de la phase de remplissage chez 14 génotypes de blé dur (*Triticum durum* Desf.) en zone semi-aride. [Rate and duration of the filling phase of 14 durum wheat genotypes (*Triticum durum* Desf.) in semi-arid zone]. *Recherche agronomique, INRAA*, **3**, 9–17.

Adamski, T. (1979): The obtaining of autodiploid barley lines using haploids from the cross *Hordeum vulgare* L. × *Hordeum bulbosum* L. *Genet. Pol.*, **20**, 31–41.

Ali, N., Javidfar, F., Mirza, Y. (2003): Selection of stable rapeseed (*Brassica napus* L.) genotypes through regression analysis. *Pak. J. Bot.*, **35**, 175–183.

Annicchiarico, P. (2002): Genotype × environment interactions – Challenges and opportunities for plant breeding and cultivar recommendations. *FAO Plant Production and Protection Paper 174*, Rome, pp. 30–33.

Avis, J. D., Taliaferro, C. M., Holbert, D. (2009): Genotype × environment interaction study of bermudagrass yields in Oklahoma. *Proc. Okla. Acad.*, **60**, 69–74.

Bagga, A. K., Buwali, K. N., Asana, R. D. (1970): Comparison of response of some Indian and semi-dwarf Mexican wheats to unirrigated cultivation. *Indian J. Agric. Sci.*, **40**, 421–427.

Bahlouli, F., Bouzerzour, H., Benmahammed, A. (2001): Etude de la réponse à la sélection de la précocité chez l'orge (*Hordeum vulgare* L.) en zones semi-arides d'altitude. [Study of the response to selection for precocity in barley (*Hordeum vulgare* L.) in high altitude semi-arid zones]. *Annales de l'Institut National Agronomique, El-Harrach, Alger*, **22**, 1–25.

Baker, R. J. (1988): Analysis of genotype-environmental interactions in crops. *ISI Atlas of Animal and Plant Sci.*, **1**, 1–4.

Becker, H. C., Leon, J. (1988): Stability analysis in plant breeding. *Plant Breeding*, **101**, 1–23.

Ben Abdallah, N., Bensalem, M. (1993): Paramètres morphophysiologiques de sélection pour la résistance à la sécheresse des céréales. (Morphophysiological selection parameters for drought resistance in cereals). In: Monneveux, P., Belhassen, E. (eds.), *Tolérance à la sécheresse des céréales en zones méditerranéenne: diversité génétique et amélioration variétale.* INRA, Montpellier (France), 15–17 décembre 1992. Les colloques 64, pp. 83–99.

Bouzerzour, H. (2000): Stabilité des performances et caractéristiques phénomorphologiques de quelques variétés de blé dur issues d'une sélection multilocale. (Stability of the performance and phenomorphological characteristics of some varieties of durum wheat resulting from multilocation selection.) *Actes du premier symposium international sur la filière blé: blé 2000. Enjeu et stratégie.* OAIC, Alger (Algérie), 7– 9 février 2000, pp. 187–196.

Bouzerzour, H., Djekoun, A., Benmahammed, N., Hassous, L. (1996): Contribution de la biomasse, l'indice de récolte et la précocité au rendement de l'orge (*Hordeum vulgare* L.) en zones semi-arides. [Contribution of the biomass, the harvest index and precocity to the barley yield (*Hordeum vulgare* L.) in semi-arid zones]. *Annales de l'Institut National Agronomique, El-Harrach, Alger*, **17**(2), 193–199.

Bouzerzour, H., Monneveux, P. (1993): Analyse des facteurs de stabilité du rendement de l'orge dans les conditions de hauts plateaux de l'est algérien. (Analysis of factors influencing the stability of barley yields under the conditions on the high plains of the Algerian east.) In: Monneveux, P., Belhassen, E. (eds.), *Tolérance à la sécheresse des céréales en zones méditerranéenne: diversité génétique et amélioration variétale.* INRA, Montpellier (France), 15–17 décembre 1992. Les colloques 64, pp. 139–158.

Debaek, P., Puech, J., Casals, M. L. (1996): Elaboration du rendement du blé d'hiver en condition de déficit hydrique. Etude en lysimètres. (Development of the yield of winter wheat under moisture stress conditions. Study in lysimeters.). *Agronomie*, **16**, 3–23.

Djebrani, M. (2000): Adaptation au déficit hydrique de quatre variétés de blé dur. (Adaptation to moisture stress of four varieties of durum wheat.) *Actes du premier symposium international sur la filière blé: blé 2000. Enjeu et stratégie.* OAIC, Alger (Algérie), 7–9 février 2000, pp. 161–170.

Eberhart, S. A., Russell, W. A. (1966): Stability parameters for comparing varieties. *Crop Sci.*, **1**, 36–40.

Finlay, K. W., Wilkinson, G. N. (1963): The analysis of adaptation in a plant-breeding programme. *Aust. J. Agric. Res.*, **14**, 742–754.

Gallais, A. (1990): *Théorie de la sélection en amélioration des plantes*. (Theory of selection in the improvement of plants). Collection Sciences Agronomiques, Masson, Paris.

Gate, P., Bouthier, A., Casabianca, H., Deleensa, E. (1993): Caractères physiologiques décrivant la tolérance à la sécheresse des blés cultivés en France: Interprétation des corrélations entre le rendement et la composante isotopique du carbone des grains. (Physiological characters describing the drought tolerance of wheat cultivated in France: Interpretation of the correlations between the grain yield and the carbon isotope component of the grains.) In: Monneveux, P., Belhassen, E. (eds.), *Tolérance à la sécheresse des céréales en zones méditerranéenne: diversité génétique et amélioration variétale.* INRA, Montpellier (France), 15–17 décembre 1992. Les colloques 64, pp. 61–73.

Gate, P., Bouthier, A., Monnier, J. L. (1992): La tolérance des variétés à la sécheresse: une réalité à valoriser. (The tolerance of varieties to drought: a reality to be developed.) *Perspectives agricoles*, **169**, 62–67.

Hanifi, L. (1999): *Contribution à l'étude de l'hétérosis et de l'intérêt des F_1, F_2 et lignées haploïdes doublées chez l'orge.* (Contribution to the study of heterosis and the interest of F_1, F_2 and doubled haploid lines in barley.) Thèse de Doctorat des Sciences Biologie, USTL, Lille, France, 177 p.

Kasha, K. J., Kao, K. N. (1970): High frequency haploid production in barley. *Nature*, **225**, 874–876.

Mekliche, A., Gate, P., Bouthier, A. (1993): Analyse comparative des comportements à la sécheresse du blé tendre et du blé dur. (Comparative analysis of the behaviour of common wheat and durum wheat to drought.) In: Monneveux P., Belhassen, E. (eds.) *Tolérance à la sécheresse des céréales en zones méditerranéenne: diversité génétique et amélioration variétale.*, INRA, Montpellier (France), 15–17 décembre 1992. Les colloques, 64, pp. 299–309.

Perkins, J. M., Jinks, J. L. (1968): Environmental and genotype × environmental components of variability III. Multiple inbred lines and crosses. *Heredity*, **23**, 339–356.

Pinthus, M. J. (1973): Estimates of genotypic value: a proposed method. *Euphytica*, **22**, 345–351.

Shukla, G. K. (1972): Some statistical aspects of portioning genotype-environmental components of variability. *Heredity*, **29**, 237–245.

Tarakanovas, P., Ruzgas, V. (2006): Additive main effect and multiplicative interaction analysis of grain yield of wheat varieties in Lithuania. *Agronomy Research*, **4**, 91–98.

Ukai, Y. (2000): Analysis of the interaction between genotypes and environment. http://lbm.ab.a.u-tokyo.ac.jp/~ukai/gest98.html

Wachira, F., Wilson, N. G., Omolo, J., Mamati, G. (2002): Genotype × environment interactions for tea yields. *Euphytica*, **127**, 289–296.

Wricke, G. (1962): Über eine Methode zur Erfassung der ökologischen Streubreite in Feldversuchen. (A method for determining ecological deviation in field experiments.) *Z. Pflanzenzucht.*, **47**, 92–96.

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AGRONOMIC PERFORMANCE OF SEVEN PEA (*Pisum sativum*) GENOTYPES WITH FIVE SOWING DATES IN SANDY SOIL

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A field experiment was conducted at the Agriculture Faculty Farm of Sebha University during the 2007/2008 and 2008/2009 winter seasons to study the agronomic performance of seven pea genotypes with different sowing dates in sandy soil. The experiment was laid out in a randomized complete block design (RCBD) using a split-plot arrangement with three replicates. The five sowing dates (30 October, 15 November, 30 November, 15 December and 30 December) were assigned to the main plots, while the seven pea genotypes (Ambassador, Pollon, MG130256, G22765-2c, 89-P-109-11, No. 252, Victory Freezer and Master B) were allocated to the sub-plots. The sowing dates had a significant effect on all the studied traits except seed protein content in both seasons. Early sowing (15 November) was better than the other sowing dates for all the traits except 100-seed weight. There were significant differences between the pea genotypes for all the traits in the two growing seasons. The Victory Freezer genotype surpassed the other genotypes for all traits except number of branches plant^{-1} in the second season, 100-seed weight and seed protein content. The highest values for number of branches plant^{-1} in the second season and for seed protein content were obtained for the G22765-2c genotype, while the maximum values of 100-seed weight were recorded for the MG130256 genotype. A significant interaction between sowing dates and pea genotypes was detected for the length of the period from emergence to initial flowering, number of pods plant^{-1}, seed yield plant^{-1} and seed yield ha^{-1} in both seasons. The longest period from emergence to initial flowering was obtained for the Victory Freezer pea variety sown on 30 November, while the highest values of pods plant^{-1}, seed yield plant^{-1} and seed yield ha^{-1} were gained by sowing the Victory Freezer pea genotype on 15 November.

Key words: sowing date, agronomic performance, *Pisum sativum* L.

Introduction

Peas are considered to be an important legume crop throughout the world, and their productivity is dependent on sowing date and genotype interaction as well as on agronomic practices. Photoperiod and temperature are major

environmental factors that change from day to day and their effects are reflected in the growth, flowering and seed maturity of pea. The choice of a suitable sowing date thus means suitable weather conditions for pea genotypes, leading to higher yields. Any decrease in temperature to below 15°C during the germination or vegetation stages retards metabolic processes and leads to seed abortion or poorer flower set compared to temperatures of 15–20°C (Gubbles, 1977). The planting date was found to affect yield, yield components and the length of reproductive phases in pea (Fletcher et al., 1966; Murray et al., 1984; Alsadon and Khalil, 1994). The early or late planting of winter pea led to a significant decrease in the seed yield (Knott and Belcher, 1998). The temperature prevailing in the growing season had a significant effect on germination, flowering, pod formation and filling, and the length of the growing season (Summerfield and Roberts, 1985; Domulin and Eteve, 1994). Pea genotypes varied widely between locations and planting dates. Yield may vary greatly due to the environmental conditions at the time of flowering (Fletcher et al., 1966; Hardwick et al., 1979). On the other hand, although Alsadon and Khalil (1994) found significant differences between two pea cultivars in yield components, there were insignificant differences in total yield, because each cultivar showed superiority in one yield component and inferiority in another, which equalled out in terms of total yield. Poggio et al. (2005) reported cultivar differences in aboveground biomass, seed yield, and pod and seed numbers m^{-2}. Because pea cultivars exhibit various sensitivities to environmental conditions (e.g. weather conditions, photoperiod), it is hypothesized that sowing date and cultivar should influence seed yield.

The objective of this research was to study the agronomic performance of seven pea genotypes at different sowing dates.

Materials and methods

A field experiment was conducted at the Agriculture Faculty Farm of Sebha University during the 2007/2008 and 2008/2009 winter seasons to study the agronomic performance of seven pea genotypes at different sowing dates in sandy soil. The soil of the experimental site was sandy, comprising 92.52% sand, 5.48% silt and 3.0% clay, with a pH of 7.8 and EC 1.2 dS m^{-1}. The experiment was laid out in a randomized complete block design (RCBD) with a split-plot arrangement and three replicates. The five sowing dates (30 October, 15 November, 30 November, 15 December and 30 December) were assigned to the main plots, while the sub-plots were allocated to the seven pea genotypes (Ambassador, Pollon, MG130256, G22765-2c, 89-P-109-11, No. 252, Victory Freezer and Master B), obtained from a local seed firm. The plot size was 10.5 m^2. The seeds were hand planted in rows 50 cm apart, with 20 cm between hills. The plants were thinned to one plant per hill three weeks after planting. Di-ammonium phosphate (18% N and 46% P_2O_5) at a rate of 173.91 kg/ha was applied during soil preparation and 105 kg urea/ha (46.0% nitrogen) was added at 50% flowering. All other cultural practices were carried out as recommended for pea production in both seasons. The means and ranges of temperature and relative humidity during the two growing seasons are shown in Table 1. The length of the period from emergence to the start of flowering was calculated according to Tawaha and Turk (2004), after which heat units were calculated.

Table 1
Temperature (°C) and relative humidity (%) data recorded during the 2007/2008 and 2008/2009 growing seasons

Periods		2007/2008			2008/2009		
		Maximum temperature	Minimum temperature	Relative humidity	Maximum temperature	Minimum temperature	Relative humidity
Oct	11–20	36.22	13.82	18.41	33.22	14.06	20.12
	21–31	33.88	13.41	18.96	30.38	13.11	19.11
Nov	1–10	30.66	16.51	21.06	27.88	12.27	22.17
	11–20	29.18	11.33	20.82	24.96	12.63	21.77
	21–30	28.79	13.49	20.97	24.98	11.93	23.82
Dec	1–10	25.77	11.10	36.22	21.42	10.33	40.12
	11–20	23.29	8.22	35.06	18.81	9.68	38.44
	21–31	20.04	8.52	35.11	16.68	6.66	39.12
Jan	1–10	19.94	7.71	40.88	14.45	6.26	44.12
	11–20	19.88	7.41	40.16	14.63	4.82	43.43
	21–31	20.61	6.66	42.28	17.16	2.61	41.21
Feb	1–10	21.55	8.77	42.70	19.88	6.55	40.36
	11–20	23.73	9.66	45.73	18.66	3.22	43.21
	21–28	22.68	8.82	46.22	19.81	2.27	51.36
Mar	1–10	27.11	8.58	42.61	23.83	7.66	40.31
	11–20	24.03	11.65	38.33	21.91	8.66	42.33
	21–31	24.33	12.37	41.16	24.22	9.37	42.36
Apr	1–10	28.77	14.96	36.22	28.37	12.22	40.18
	11–20	34.88	15.67	31.26	31.67	13.21	36.82
	21–30	35.42	15.13	28.28	30.15	12.77	31.27

Source: Meteorological data station at Sebha International Airport

At harvest, ten guarded plants were sampled randomly from each experimental unit and the number of branches and pods plant^{-1}, number of seeds pod^{-1}, 100-seed weight (g) and seed yield plant^{-1} (g) were recorded. The seed yield was determined for each sub-plot, then converted to kg ha^{-1}. A seed sample of 25 g from each sub-plot was milled using a 20-mesh laboratory Willy mill. The nitrogen content was determined with the semi-microKjeldahl method to estimate the crude protein % (A.O.A.C., 1980).

All data collected were analysed with analysis of variance (ANOVA). Differences between means were compared by LSD at the 5% level of significance (Gomez and Gomez, 1984).

Results and discussion

Days from sowing to the start of flowering; heat units

The data in Tables 2 and 3 revealed that the sowing date had a significant effect ($p \leq 0.05$) on the length of the period from sowing to the start of flowering and on the heat units required in both seasons. This may be ascribed to the effect of the temperature prevailing at sowing on germination, which was high in early sowing and low in late sowing (Table 1). The period from emergence to the start of flowering was decreased by planting dates earlier or later than 30 November in both growing seasons. Pea sown on 30 December had a lower heat unit

requirement than the other planting dates for the period from sowing to the start of flowering, being 889.05°C and 931.78°C in the 2007/2008 and 2008/2009 seasons, respectively. This trend could be ascribed to the low temperature associated with the 30 December sowing date (Table 1) and the shortening of the growth stages. This is supported by the studies conducted by Summerfield and Roberts (1985) and Domulin and Eteve (1994).

There were significant differences between the genotypes for all the traits in the two growing seasons (Tables 2–9). The Victory Freezer variety surpassed the other varieties in the length of the period from planting to the start of flowering (56.27 and 75.61 days in the first and second seasons, respectively). This could be due to a combination of genotypic behaviour and environmental conditions. Similar findings were reported by Poggio et al. (2005), who stated that pea cultivars exhibit various sensitivities to environmental conditions. Victory Freezer had the highest heat unit requirement, because it had a long period from sowing to the start of flowering.

The interaction between pea genotypes and sowing dates had a significant influence ($p \leq 0.05$) on the length of the period from sowing to the start of flowering in the two growing seasons, the highest values being obtained for Victory Freezer sown on 30 November.

Table 2

Effect of sowing dates, genotypes and their interaction on the length of the period from planting to the start of flowering (days)

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	52.53	50.02	61.40	47.45	35.83	49.44
	Pollon	43.48	47.04	53.88	44.44	34.21	44.61
	MG130256	59.69	57.10	67.00	58.01	38.19	55.99
	G22765-2c	37.17	35.02	46.03	41.00	28.67	37.57
	89-P-109-11, No. 252	52.14	52.12	61.62	46.02	34.77	49.33
	Victory Freezer	58.71	57.22	67.12	58.22	40.12	56.27
	Master B	39.12	36.12	47.12	40.66	29.66	38.53
	Mean	48.97	47.80	57.73	47.97	34.49	
2008/2009	Ambassador	62.50	55.40	65.00	53.45	39.79	55.22
	Pollon	52.47	50.58	59.00	52.00	38.43	50.49
	MG130256	65.69	61.82	72.02	66.03	42.23	61.55
	G22765-2c	45.36	39.80	48.41	49.00	34.01	43.31
	89-P-109-11, No. 252	64.00	57.30	65.64	52.60	40.77	56.06
	Victory Freezer	46.71	60.83	73.11	66.00	44.12	61.75
	Master B	43.41	40.63	48.11	49.66	35.12	43.38
	Mean	56.87	52.33	61.61	55.53	39.21	
L.S.D. 0.05 for sowing dates			7.41			4.51	
L.S.D. 0.05 for genotypes			5.72			5.83	
L.S.D. 0.05 for interaction			10.61			9.87	

Table 3
Effect of sowing dates, genotypes and their interaction on the heat requirements for the period from emergence to the start of flowering

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	1039.90	1027.26	955.82	1046.44	879.44	989.76
	Pollon	868.22	977.82	961.71	878.62	850.88	907.45
	MG130256	1226.66	1192.00	1078.06	1132.78	950.18	1115.93
	G22765-2c	862.14	901.70	867.71	861.83	846.40	867.95
	89-P-109-11, No. 252	1091.62	1064.88	956.77	978.06	883.11	994.88
	Victory Freezer	1223.33	1192.07	1080.11	1132.88	956.11	1116.90
	Master B	863.48	904.12	866.88	861.11	857.23	870.56
	Mean	1024.90	1037.11	966.72	984.53	889.05	
2008/2009	Ambassador	1118.94	1097.20	995.62	1106.44	921.15	1047.86
	Pollon	967.22	1017.80	1011.71	936.62	894.18	965.50
	MG130256	1306.66	1242.00	1138.11	1192.14	994.17	1174.61
	G22765-2c	902.20	951.04	916.25	911.11	842.18	914.55
	89-P-109-11, No. 252	1151.62	1114.68	996.74	1025.22	929.22	1043.49
	Victory Freezer	1305.82	1245.11	1140.16	1192.00	995.77	1175.77
	Master B	906.33	951.93	920.11	912.00	895.81	917.23
	Mean	1094.11	1088.53	1016.95	1039.36	931.78	
L.S.D. 0.05 for sowing dates			63.44			72.73	
L.S.D. 0.05 for genotypes			119.41			120.82	
L.S.D. 0.05 for interaction			NS			NS	

NS: Non-significant

Yield attributes

The number of branches and pods plant^{-1}, seed number pod^{-1}, 100-seed weight and seed yield plant^{-1} responded significantly ($p \leq 0.05$) to the sowing dates (Tables 4–8). The highest numbers of branches plant^{-1} (4.67 and 5.31 in 2007/2008 and 2008/2009, respectively) were registered for the 15 November sowing date, and the lowest (3.17 and 3.75 for the two seasons) for the 30 December sowing date. The reduction in number of branches after late sowing can be explained by the short period from emergence to the start of flowering (Table 2), which had a negative effect on carbohydrate accumulation and consequently on the number of branches. Delaying sowing from 30 October to 30 December also drastically decreased the number of pods per plant in both seasons, except for the mid-November sowing date in the second season only. The superiority of early sowing for the number of pods plant^{-1} can be explained by the larger number of branches plant^{-1}. The data also revealed the significant effect of sowing date on the number of seeds pod^{-1} in the two growing seasons. Mid-November sowing surpassed the other sowing dates in this respect and gave the highest values of seeds pod^{-1} (5.51 and 6.21 in 2007/2008 and 2008/2009, respectively). This can be ascribed to the suitable conditions prevailing during pollination and fertilization after sowing on 15 November, which led to an increase in seed formation compared to the other sowing dates.

The 100-seed weight was also significantly affected by the sowing date in both seasons. The heaviest seeds (17.06 and 20.12 g) were obtained from the 30 November and 30 December sowing dates in the 2007/2008 and 2008/2009 seasons, respectively. This was to be expected, since late sowing reduced the number of seeds per pod, which led to less competition for metabolites between the seeds in each pod, consequently resulting in heavier seeds. The highest values of seed yield plant^{-1} were obtained for the 15 November sowing date. This could be ascribed to the same trend obtained for number of pods plant^{-1} and number of seeds pod^{-1}.

The data proved the significant effect of the genotype on the traits investigated in both growing seasons. The maximum values of number of branches and pods plant^{-1} were recorded for Victory Freezer in the first season (4.87 and 31.37, respectively) and for G22765-2c in the second season (5.38 and 36.79, respectively), with no significant difference between the two genotypes. The highest values of 100-seed weight (16.64 and 19.86 g in the first and second seasons, respectively) were obtained for the MG130256 genotype, while Victory Freezer had the highest values of seed yield per plant (32.89 and 35.93 g/plant in 2007/2008 and 2008/2009, respectively). These results are in good agreement with those obtained by Fletcher et al. (1966), Murray et al. (1984) and Alsadon and Khalil (1994). Here too, the interaction between sowing dates and genotypes had a significant effect on the number of pods and seed yield per plant in both seasons.

The highest values of number of pods plant^{-1} (35.17 and 42.61 in the first and second seasons, respectively) and seed yield per plant (38.72 and 44.81 g/plant, respectively) were registered for the Victory Freezer genotype planted on 30 October and 15 November in the 2007/2008 and 2008/2009 seasons, respectively.

Table 4
Effect of sowing dates, genotypes and their interaction on the number of branches/plant

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	2.30	3.30	3.11	2.91	2.32	2.98
	Pollon	3.20	3.90	2.90	3.07	2.50	3.11
	MG130256	5.30	5.20	4.93	4.40	3.66	4.69
	G22765-2c	5.30	5.41	5.21	4.51	3.80	4.84
	89-P-109-11, No. 252	60.4	4.31	4.03	3.59	2.72	3.85
	Victory Freezer	5.37	5.48	5.23	4.49	3.78	4.87
	Master B	4.78	5.09	4.92	4.33	3.43	4.51
	Mean	4.55	4.67	4.33	3.90	3.17	
2008/2009	Ambassador	3.90	3.92	3.51	3.23	2.94	3.50
	Pollon	3.80	4.32	3.24	3.49	3.18	3.60
	MG130256	5.80	5.82	29.5	4.84	4.34	5.21
	G22765-2c	5.92	5.97	5.71	4.95	4.36	5.38
	89-P-109-11, No. 252	5.20	4.97	4.39	4.07	3.28	4.38
	Victory Freezer	5.87	6.31	4.98	4.86	4.29	5.26
	Master B	5.71	5.86	4.86	4.68	3.87	4.99
	Mean	5.17	5.31	4.56	4.30	3.75	
L.S.D. 0.05 for sowing dates			0.65			0.81	
L.S.D. 0.05 for genotypes			0.32			0.29	
L.S.D. 0.05 for interaction			NS			NS	

NS: Non-significant

Table 5
Effect of sowing dates, genotypes and their interaction on the number of pods/plant

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	24.66	24.10	21.11	16.75	12.13	19.74
	Pollon	34.11	26.09	24.13	18.94	12.45	23.14
	MG130256	30.10	29.86	30.92	26.66	25.24	28.55
	G22765-2c	32.00	32.81	31.22	27.57	26.26	29.96
	89-P-109-11, No. 252	98.18	19.95	16.39	13.65	8.25	15.44
	Victory Freezer	35.17	35.14	33.12	27.14	26.32	31.37
	Master B	29.28	31.28	26.14	26.41	21.28	26.87
	Mean	29.18	28.46	26.14	22.44	18.84	
2008/2009	Ambassador	30.87	29.32	25.42	22.87	17.31	25.15
	Pollon	11.31	33.27	30.41	24.22	18.36	27.47
	MG130256	36.23	37.14	38.00	33.21	31.00	35.11
	G22765-2c	39.27	40.12	38.41	34.17	32.02	36.79
	89-P-109-11, No. 252	25.12	26.23	22.00	19.36	13.46	21.23
	Victory Freezer	38.38	42.61	36.41	34.00	29.16	36.11
	Master B	38.09	38.21	34.07	32.78	25.34	33.00
	Mean	34.15	35.27	32.10	28.65	23.80	
L.S.D. 0.05 for sowing dates			3.72			3.42	
L.S.D. 0.05 for genotypes			2.91			2.11	
L.S.D. 0.05 for interaction			7.58			5.57	

Table 6
Effect of sowing dates, genotypes and their interaction on the number of seeds/pod

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	5.56	6.30	5.05	4.20	3.66	4.95
	Pollon	4.88	4.96	3.99	3.32	2.56	3.94
	MG130256	4.61	4.78	4.47	3.49	3.26	4.12
	G22765-2c	4.28	4.88	4.25	3.85	3.49	4.15
	89-P-109-11, No. 252	5.52	6.12	4.82	4.14	3.25	4.77
	Victory Freezer	4.81	6.28	4.82	4.09	3.57	4.71
	Master B	4.69	5.24	4.18	3.89	3.32	4.26
	Mean	4.90	5.51	4.51	3.85	3.30	
2008/2009	Ambassador	6.31	7.12	5.93	4.98	4.41	5.75
	Pollon	5.76	5.72	4.83	4.11	3.27	4.73
	MG130256	5.22	5.53	5.22	4.21	4.00	4.83
	G22765-2c	5.11	5.66	5.00	4.56	4.21	4.90
	89-P-109-11, No. 252	6.18	6.53	5.66	4.87	4.03	5.45
	Victory Freezer	6.41	6.93	6.07	4.91	4.24	5.71
	Master B	5.31	6.01	5.08	4.72	4.08	5.04
	Mean	5.75	6.21	5.39	4.62	4.03	
L.S.D. 0.05 for sowing dates			1.12			1.01	
L.S.D. 0.05 for genotypes			NS			NS	
L.S.D. 0.05 for interaction			NS			NS	

NS: Non-significant

Table 7
Effect of sowing dates, genotypes and their interaction on 100-seed weight (g)

Seasons	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	15.22	13.92	13.72	16.50	15.96	15.06
	Pollon	14.22	15.20	18.90	15.95	17.00	16.25
	MG130256	16.42	15.33	17.87	16.69	16.93	16.64
	G22765-2c	16.15	14.67	17.62	16.56	17.07	16.41
	89-P-109-11, No. 252	12.00	10.56	16.16	16.22	17.46	14.48
	Victory Freezer	13.82	14.08	17.68	15.92	16.87	15.67
	Master B	15.72	14.14	17.52	16.09	16.88	16.07
	Mean	14.79	13.98	17.06	16.27	16.88	
2008/2009	Ambassador	18.89	17.41	17.06	19.69	19.00	18.40
	Pollon	17.93	18.61	03.22	19.16	20.41	19.62
	MG130256	19.63	18.74	21.01	19.79	20.18	19.86
	G22765-2c	19.61	18.12	20.84	19.74	20.21	19.70
	89-P-109-11, No. 252	15.21	14.00	19.41	19.31	20.77	17.74
	Victory Freezer	18.47	18.02	18.71	19.67	20.51	19.07
	Master B	18.17	17.83	17.71	19.49	19.78	18.59
	Mean	18.26	17.53	19.53	19.54	20.12	
L.S.D. 0.05 for sowing dates			1.73			1.24	
L.S.D. 0.05 for genotypes			0.48			0.68	
L.S.D. 0.05 for interaction			NS			NS	

NS: Non-significant

Table 8
Effect of sowing dates, genotypes and their interaction on seed yield/plant (g)

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	28.37	32.13	21.98	17.34	10.64	22.09
	Pollon	26.39	32.73	27.52	15.17	9.61	22.28
	MG130256	33.35	33.88	36.74	23.43	21.50	29.78
	G22765-2c	62.34	36.45	35.34	26.60	96.22	31.19
	89-P-109-11, No. 252	20.51	20.51	19.42	12.64	7.76	16.16
	Victory Freezer	38.72	38.12	36.14	27.41	24.08	32.89
	Master B	34.82	33.92	32.81	25.71	21.89	29.83
	Mean	30.96	32.53	29.99	21.18	16.92	
2008/2009	Ambassador	32.17	36.11	66.25	20.74	14.02	25.74
	Pollon	30.09	33.33	31.19	18.68	12.11	25.08
	MG130256	37.02	37.55	40.41	27.01	25.06	33.41
	G22765-2c	38.44	40.06	39.00	30.11	26.44	34.81
	89-P-109-11, No. 252	24.00	24.14	23.01	16.31	11.31	19.75
	Victory Freezer	39.71	44.81	38.61	30.67	25.88	35.93
	Master B	37.06	38.56	34.15	28.48	21.07	31.86
	Mean	34.07	36.36	33.14	24.57	19.41	
L.S.D. 0.05 for sowing dates			6.41			7.06	
L.S.D. 0.05 for genotypes			4.19			5.31	
L.S.D. 0.05 for interaction			11.01			12.86	

Seed yield

The data in Table 9 revealed that the sowing date had a significant effect ($p \leq 0.05$) on the seed yield ha^{-1} in both growing seasons. A linear reduction in seed yield was observed when delaying the sowing date from 15 November to 30 December. This could be ascribed to the reduction in yield components in late sowing date treatments, which led to lower seed yield ha^{-1}, as also reported by Knott and Belcher (1998) and Hatice et al. (2007).

Pea genotypes varied significantly ($p \leq 0.05$) in seed yield ha^{-1} in both seasons (Table 9), with the highest mean values (3289.00 and 3593.00 kg/ha in the 2007/2008 and 2008/2009, respectively) for Victory Freezer and the lowest for 89-P-109-11, No. 252, the genotype that had the lowest values for number of pods/plant, 100-seed weight and seed yield per plant. These results are in good agreement with those obtained by Alsadon and Khalil (1994) and Poggio et al. (2005).

The interaction between pea genotypes and sowing dates was significant in both seasons, the maximum seed yield (3872.00 and 4481.00 kg/ha in the 2007/2008 and 2008/2009 growing seasons, respectively) being achieved by sowing Victory Freezer on 15 November. This could be ascribed to the significant interactions for number of branches and pods $plant^{-1}$, number of seeds pod^{-1} and seed yield $plant^{-1}$.

Table 9
Effect of sowing dates, genotypes and their interaction on seed yield ha^{-1}

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	2837.00	3213.00	2198.00	1734.00	1064.00	2209.00
	Pollon	2639.00	3273.00	2752.00	1517.00	961.00	2228.00
	MG130256	3335.00	3388.00	3674.00	2343.00	2150.00	2978.00
	G22765-2c	3462.00	3645.00	3534.00	2660.00	2296.00	3119.00
	89-P-109-11, No. 252	2051.00	2051.00	1942.00	1264.00	776.00	1616.00
	Victory Freezer	3872.00	3812.00	3614.00	2741.00	2408.00	3289.00
	Master B	3482.00	3392.00	3281.00	2571.00	2189.00	2983.00
	Mean	3096.00	3253.00	2999.00	2118.00	1692.00	
2008/2009	Ambassador	3217.00	3611.00	2566.00	2074.00	1402.00	2574.00
	Pollon	3009.00	3333.00	3119.00	1868.00	1211.00	2508.00
	MG130256	3702.00	3755.00	4041.00	2701.00	2506.00	3341.00
	G22765-2c	3844.00	4006.00	3900.00	3011.00	2644.00	3481.00
	89-P-109-11, No. 252	2400.00	2414.00	2301.00	1631.00	1131.00	1975.00
	Victory Freezer	3971.00	4481.00	3861.00	3067.00	2588.00	3593.00
	Master B	3706.00	3856.00	3415.00	2848.00	2107.00	3186.00
	Mean	3407.00	3636.00	3314.00	2457.00	1941.00	
L.S.D. 0.05 for sowing dates			64.1			70.6	
L.S.D. 0.05 for genotypes			41.9			53.1	
L.S.D. 0.05 for interaction			110.1			128.6	

Seed protein content (%)

The data in Table 10 illustrate the significant influence ($p \leq 0.05$) of sowing date and pea genotype on seed protein content, with maximum values for the genotype G22765-2c (23.58% in 2007/2008, 22.84% in 2008/2009). This may be due to the genetic background and to its interaction with environmental factors.

Table 10
Effect of sowing dates, genotypes and their interaction on seed protein content (%)

Season	Genotype	Sowing date					Mean
		30 Oct	15 Nov	30 Nov	15 Dec	30 Dec	
2007/2008	Ambassador	20.00	20.12	20.16	20.21	19.12	19.92
	Pollon	21.27	22.28	22.68	21.87	20.77	21.77
	MG130256	23.85	23.87	23.88	23.24	23.00	23.56
	G22765-2c	23.78	23.78	23.81	23.41	23.12	23.58
	89-P-109-11, No. 252	19.78	19.21	19.82	19.26	18.72	19.35
	Victory Freezer	22.72	22.38	22.78	22.17	22.17	22.44
	Master B	22.89	21.72	22.58	22.29	22.23	22.34
	Mean	22.04	21.90	22.24	21.77	21.30	
2008/2009	Ambassador	19.20	19.31	19.35	19.50	18.37	19.14
	Pollon	20.51	21.50	21.90	21.16	19.99	21.01
	MG130256	23.11	23.11	23.14	22.56	22.22	22.82
	G22765-2c	23.00	23.06	23.06	22.78	22.33	22.84
	89-P-109-11, No. 252	18.98	18.43	19.09	18.54	18.00	18.60
	Victory Freezer	21.46	22.18	22.11	21.18	22.18	21.82
	Master B	21.55	22.47	22.31	22.08	22.21	22.12
	Mean	21.11	21.43	21.56	21.11	20.75	
L.S.D. 0.05 for sowing dates			NS			NS	
L.S.D. 0.05 for genotypes			1.86			1.93	
L.S.D. 0.05 for interaction			NS			NS	

NS: Non-significant

Conclusions

Based on the results the authors recommend sowing the Victory Freezer pea variety on 15 November in order to obtain the highest seed yield.

References

Alsadon, A. A., Khalil, S. O. (1994): Yield and yield components analysis of pea cultivars in response to planting date. *Alex. J. Agric. Res.*, **39**, 471–489.

A.O.A.C. (1980): *Official Methods of Analysis*, 13th ed. Association of Official Agricultural Chemists. Washington, D.C.

Domulin, V. N., Eteve, G. (1994): Variability of seed and plant development in pea. *Crop Sci.*, **634**, 992–998.

Fletcher, H. F., Orrarod, D. P., Maurer, A. R., Stanfield, B. (1966): Response of peas to environment. I. Planting date and location. *Can. J. Plant Sci.*, **46**, 77–85.

Gomez, K. N., Gomez, A. A. (1984): *Statistical Procedures for Agricultural Research*, 2nd ed. John Wiley and Sons Inc., New York.

Gubbles, G. H. (1977): Quality, yield and weight per seed of green field peas as affected by sowing and harvest date. *Can. J. Plant Sci.*, **57**, 1029–1032.

Hardwick, R. C., Andrews, D. J., Hole, C. C., Salter, P. J. (1979): Variability in number of pods and yield in commercial crops of veining peas (*Pisum sativum* L.). *J. Agric. Sci., Camb.*, **92**, 675–681.

Hatice, B., Erkut, P., Aysun, P., Ali, G. (2007): Determination of the yield performance and harvesting period of fifteen (*Pisum sativum* L.) cultivars sown in autumn and spring. *Pak. J. Bot.*, **39**, 2017–2025.

Knott, C. M., Belcher, S. J. (1998): Optimum sowing dates and plant populations for winter peas (*Pisum sativum). J. Agric. Sci.*, **131**, 449–454.

Murray, G. A., Swensen, J. B., Auld, D. L. (1984): Influence of seed size and planting date on the performance of Austrian winter field peas. *Agron. J.*, **76**, 595–598.

Poggio, S. L., Satorre, E. H., Dethiou, S., Gonzalo, G. M. (2005): Pod and seed numbers as a function of photo thermal quotient during the seed set period of field pea (*Pisum sativum* L.) crops. *Europ. J. Agron.*, **22**, 55–69.

Summerfield, R. J., Roberts, E. H. (1985): *Grain Legume Crop.* Collins, U.K., 859 p.

Tawaha, A. M., Turk, M. A. (2004): Field pea seeding management for semiarid Mediterranean conditions. *J. Agron. Crop Sci.*, **190**, 86–92.

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A SURVEY ON THE SOIL PENETRATION RESISTANCE AND SOIL MOISTURE CONTENT IN FIELD EXPERIMENTS

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Various forms of physical soil degradation, including soil compaction, have been widely investigated both in Hungary and abroad. Soil compaction is a world-scale problem, which may be triggered by both natural and artificial factors and may adversely influence the effectiveness of crop production. In the long run this unfavourable change in the physical condition of the soil may result in extra expenses, higher energy consumption and excessive environmental damage. The effect of conventional tillage on the physical condition of the soil was assessed on six farms for three years in the framework of field experiments. In this study the physical condition of the soil was examined in terms of soil penetration resistance and moisture content. Significant differences between the experiments were revealed when penetration resistance (PR) was examinated at a depth of 20–30 cm, but not at other depths. In 2004 the mean PR values exceeded 3 MPa in experiments A, B and E. In 2005 significant differences were observed between the experiments at a depth of 30–40 cm, but no adverse compaction was detected at any depth in any of the experiments. In 2006 significant differences were observed between the experiments at depths of 0–10 cm and 10–20 cm, though even in that year no adverse soil compaction was detected. In the first year significant differences in soil moisture content were revealed at depths of 10–20 cm, 20–30 cm, 30–40 cm and 40–50 cm, and in 2005 at depths of 20–30 cm and 30–40 cm. In 2006 no significant differences were found between the experiments at any depth. The highest soil moisture contents were recorded in all the experiments at a depth of 30–40 cm. All in all, the results of both penetration resistance and moisture content were indicative of favourable soil conditions. During the period investigated adversely compact layers that would hamper moisture transport were not found in any of the experiments.

Key words: conventional tillage, soil compaction, penetration resistance, soil moisture

Introduction

As a result of tillage-induced impacts, unfavourable soil conditions may appear anywhere in soil profiles used for crop production (Birkás, 2000). The reasons for the development of soil compaction and its effect on crop production

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have been widely examined in long-term field experiments both in Hungary and abroad. Rátonyi (1999) emphasized that the physical properties of the soil have a significant influence on the growth and development of cultivated plants. Due to the constant use of the soil for the same purpose, to multi-traffic tillage and to frequent soil disturbance, soil degradation processes accelerate and the soil structure deteriorates. One process involving the physical degradation of the soil is soil compaction, a worldwide problem that causes huge damage and is difficult to overcome (Birkás, 2000; Gyuricza, 2001). Deterioration in the physical condition of the soil and compaction may be caused by natural factors, as well as by human activity, but mechanization and badly performed tillage operations are primarily responsible for their occurrence (Rátonyi, 1999). Beke (2006) confirmed that soil compaction may develop due to both natural and artificial factors. The latter may be related to tillage operations performed under sub-optimum circumstances and of poor quality (Virág, 2005; Beke, 2006). In recent decades cultivation technologies have mostly involved multi-traffic crop establishment. The use of up-to-date machinery combinations that minimize the amount of traffic is rare. Deep cultivation of the soils is omitted for economic reasons, while tillage systems based on shallow primary tillage are commonly performed at the same depth as disking. Consequently even in the first year adverse compaction may be detected in the topsoil (Rátonyi, 1999). Compaction induced by vehicle traffic has adverse effects on a number of key soil properties, such as bulk density, soil resistance, porosity and hydraulic conductivity (Radford et al., 2000; Hamza and Anderson, 2005). All these factors could potentially reduce root penetration, water extraction and plant growth (Kirkegaard et al., 1992; Passioura, 2002). Evidence of reductions in crop yields as a result of soil compaction has been reported for both dryland (rainfed) cropping systems (Ellington, 1986; Radford et al., 2001; Hamza and Anderson, 2003; Sadras et al., 2005) and irrigated crops (McGarry and Chan, 1984; McGarry, 1990; Braunack et al., 1995) over a wide range of soil types and environmental circumstances (Chan et al., 2006).

According to Hungarian and foreign scientists, the soil is adversely compact if its penetration resistance in the dry (but not extremely dry) state reaches 3.0 MPa (Hakansson, 1990; Soane and van Ouwerkerk, 1994; Rátonyi, 1999; Birkás and Gyuricza, 2004). Apart from the looseness or compactness of the soil, the most important factor influencing soil resistance is the moisture content (Gyuricza, 2001).

There are significant fluctuations in the soil moisture content over both space and time (Stefanovits, 1992). Precipitation has an influence on traffic-induced compaction through the soil moisture content. Soils saturated by abundant precipitation compact easily and deeply. There is an increased risk of compaction when the precipitation falling during the cultivation and harvest period exceeds the average annual precipitation rate by $\geq$50% (Virág, 2005). Birkás and Gyuricza (2004) compared the moisture contents typical of the cultivation system over an average of 12 years in Gödöllő and over an average of 3 years in Hatvan. It was found that where the surface of the ploughed soils

was not covered by crop residues the moisture content varied depending on the method of soil preparation or the openness of the surface. It can be assumed that there is also a correlation between the moisture content and the soil conditions. Unfavourable soil conditions hamper the infiltration of precipitation and the utilization of soil water reserves. Soil moisture loss can be mitigated by improving soil moisture transport and maintaining it through proper soil conditions, minimizing the area of disturbed soils, covering the surface and optimizing the frequency of soil disturbances. Gyuricza et al. (2004) compared the ridge tillage system and conventional and zero tillage technologies under field circumstances in a long-term (eight-year) tillage experiment in Austria. The highest bulk density and penetration resistance values were recorded for zero tillage in the upper 10–20 cm layer, but in no case did the values recorded for the soils reach critical compactness levels. Even after eight years of systematic ploughing a compact, isolating layer was not formed at the tillage depth, which was attributed on the one hand to the good initial soil conditions and on the other hand to the performance of primary tillage within appropriate soil moisture ranges.

In a series of six field experiments, soil moisture content and soil resistance were investigated in a conventional tillage system. The results achieved in 2004–2006 will be reviewed in this study.

Materials and methods

Geographical location

The experiments were established in 2004 in the mid-Hungarian region (Nagykáta microregion) at six farms between Pánd (47°21'01''N, 19°38'00" E, altitude above sea level: 129 m) and Káva (47°21'19''N, 19°35'16" E, altitude above sea level: 131 m). The area is located in a valley surrounded by hills, but the experiments were laid out on a flat area.

Climate conditions

The monthly mean temperature data (Fig. 1) were measured at an automated meteorological station in the nearby Tápiószele Institute for Agrobotany. The annual mean temperature averaged over three years was 10.09°C (with a maximum monthly mean of 21.43°C in July and a minimum of –1.97°C in January). The lowest mean annual temperature values (9.81°C) were recorded in 2005.

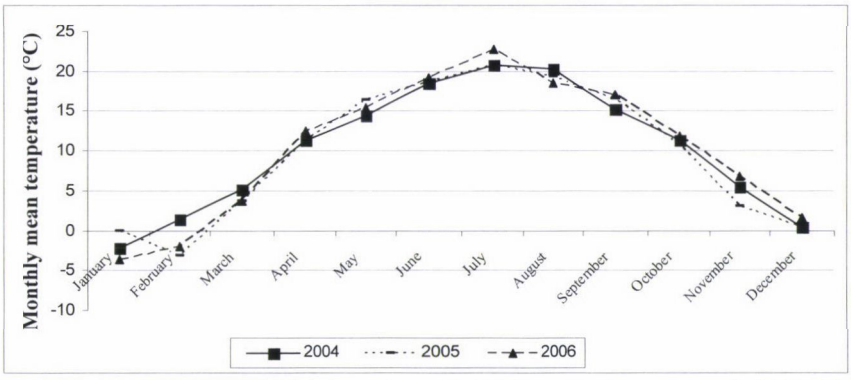

Fig. 1. Changes in mean monthly temperatures (°C) in the experimental area in 2004–2006

Precipitation data were obtained from a recording station in Nagykáta. Averaged over three years the highest monthly precipitation was recorded in June (71.20 mm) and August (99.43 mm) and the lowest (19.50 mm) in October (Fig. 2). The highest annual precipitation (702.1 mm) in the three years was received in 2005.

Soil parameters

Since no soil tests had previously been performed on the pilot farm, samples from the six experimental sites were examined for upper limit of plasticity (K_A), pH value, calcium carbonate, humus, phosphorus and potassium contents under laboratory conditions using standard procedures between 2004 and 2006. Based on the values obtained for K_A (37–39 over the three-year period) soil texture was estimated to be loam. The pH of the examined soils varied from slightly acidic to neutral (pH=5.70–7.22). The humus content in the top soil was poor (1.26–2.75%) while the AL-P_2O_5 content was 65.79–229.97 ppm and the AL-K_2O content 61.80–396.59 ppm.

The penetration resistance and soil moisture content were examined during the field experiments under conventional tillage conditions. Conventional tillage is characterized by high traffic throughput, involving time- and energy-consuming operations. The tillage depth is more frequently adjusted to the needs of the plants and the tools at hand than to the moisture or compactness of the soil. Crop residues are not utilized outside the growing period to protect and cover the soil surface, thus minimizing moisture loss. A friable seedbed free of crop residues is seen as desirable for conventional tillage. In the field experiments the same tillage systems were used for post-harvest operations in all three years: disking the upper layer was followed by autumn ploughing (30 cm). In spring, before sowing, the soil was loosened with a cultivator. Maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) were grown on the farms during the three-year period. The crop sequences in the experimental treatments were as follows: Experiment A: maize-sunflower-maize; Experiment B: maize-sunflower-maize; Experiment C: maize-maize-sunflower; Experiment D: maize-maize-maize; Experiment E: maize-maize-sunflower; Experiment F: maize-maize-maize.

Penetration resistance, soil moisture

The measurement of penetration resistance (PR), one of the most commonly used methods for investigating compaction, was applied to examine the compacted layers. Changes in the physical properties of the soil over space and time are well demonstrated if the soil moisture content is also considered. A mechanical spring-penetrometer was used in the field experiments during the vegetation period. The measurements were carried out in three replicates, at 10 cm intervals to a depth of 50 cm (Daróczi and Lelkes, 1999). The determination of soil moisture

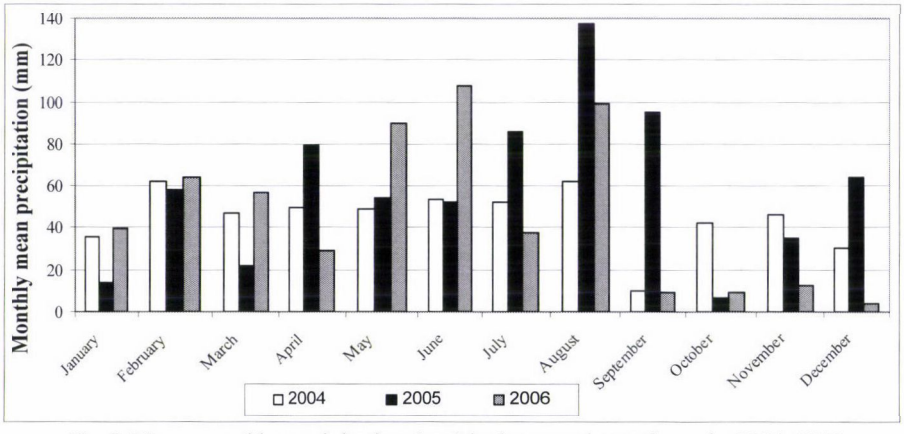

Fig. 2. Mean monthly precipitation (mm) in the experimental area in 2004–2006

content was carried out simultaneously with that of PR. The moisture content of the soil samples was determined after drying in an oven at 105°C to constant weight. Samples for soil moisture determination were again taken at 10 cm intervals to 50 cm depth in three replicates.

The PR and moisture content measurements were evaluated using the Microsoft Office Excel program. Single factor analysis of variance was applied for the statistical evaluation (Sváb, 1981; Baráth et al., 1996).

Results and discussion

The reliability of soil penetration resistance values measured using a penetrometer depends on the accuracy of the instrument, how the measurements are performed and the level of homogeneity within the experimental plot. The magnitude of the standard deviation is primarily determined by the relatively small surface of the sound cone base and by the variability of soil parameters closely related to soil penetration resistance, such as moisture content (Rátonyi, 1999). PR was determined twice during the vegetation period in 2004, and three times in 2005 and 2006, in three replications (Figs. 3–5). In the first year significant differences in PR were detected between the experiments at a depth of 20–30 cm ($LSD_{5\%}$=1.2). At the other depths no significant differences were revealed. Based on the average value of the PR measurements, the soil penetration resistance values in 2004 exceeded 3 MPa for experiments A, B and E (Fig. 3). Birkás et al. (2006) stated that the soil is adversely compact if the PR, measured with a penetrometer at a humidity equivalent to field water capacity, exceeds a value of 3.0 MPa, its bulk density is higher than 1.5 g/cm^3 and the total porosity drops below 40%. While in the case of experiments A and E PR values higher than 3.0 MPa were only recorded at a depth of 40–50 cm, in experiment B average PR values exceeding 3.4 MPa were found even at 30–40 cm. It can be concluded that adversely compact layers were found below the depth of primary tillage for three treatments in the first year examined. In all three cases the crop grown was maize. In dry soils maize roots may penetrate as deep as 2 metres. The main mass of roots, however, is located in the 30 cm upper layer of the soil, so soil penetration resistance values of >3 MPa at depths of 30–40 cm and 40-50 cm (experiment B) do not hamper plant growth. In 2005 significant differences were found at a depth of 30–40 cm ($LSD_{5\%}$=0.3), but adversely compact layers were not observed at any depth in any experiment (Fig. 4). The average PR values ranged from 0.5–2.2 MPa. Of the three years examined, 2005 proved to be the wettest, with a total precipitation of 702.1 mm, 20–23% higher than in the other two years. The favourable PR values could be attributed to tillage operations being performed in the ideal moisture range and in optimal quality. In 2006 (Fig. 5) significant differences between the experiments were found at depths of 0–10 cm ($LSD_{5\%}$=0.5) and 10–20 cm ($LSD_{5\%}$=0.6). The average PR values ranged from 0.4–2.7 MPa, so although significant differences in compaction were recorded for that year, compaction did not reach adverse levels.

On the whole it can be concluded that significant differences between the six field experiments were recorded at tillage depth in the first year, below tillage depth in the second year, and in the upper 0–20 cm in the third. Only in the first year of the experiment were values higher than 3 MPa recorded, generally accepted as indicating adverse levels of soil compaction. These were recorded only for experiments A (at 40–50 cm), B (30–40 cm and 40–50 cm) and E (40–50 cm). For none of the experiments did the average PR values reach the adverse soil compaction limit in the years 2005 and 2006. This is certainly a favourable result from a crop production point of view, since at no depths did a compact layer hamper the utilization of soil moisture, either during the rainy year of 2005 or subsequently.

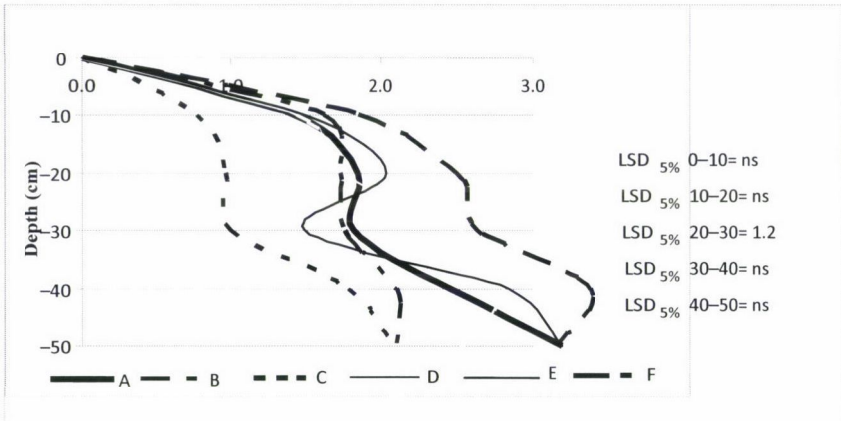

Fig. 3. Changes in penetration resistance (MPa) at depths of 0–50 cm in 2004

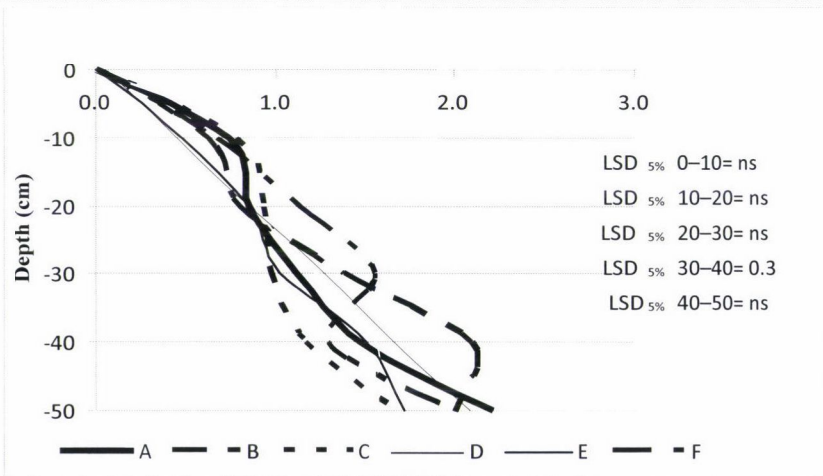

Fig. 4. Changes in penetration resistance (MPa) at depths of 0–50 cm in 2005

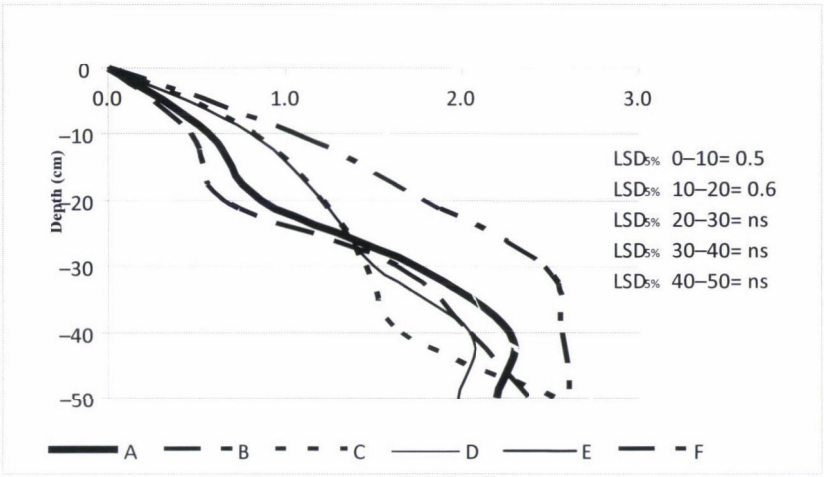

Fig. 5. Changes in penetration resistance (MPa) at depths of 0–50 cm in 2006

In order to examine the physical condition of the soil the soil moisture content was determined simultaneously with the PR measurements. Figures 6–8 show the soil moisture results to a depth of 50 cm. Gyuricza et al. (2004) pointed out that one of the major aims of tillage operations is to preserve the soil moisture, or to minimize its loss in order to ensure the undisturbed functioning of physical, chemical and biological processes. The greatest differences in soil moisture content between the experiments were recorded in 2004. In the first year of the examination (Fig. 6) significant differences were found at depths of 10–20 cm ($LSD_{5\%}$=8), 20–30 cm ($LSD_{5\%}$=7.8), 30–40 cm ($LSD_{5\%}$=7.3) and 40–50 cm ($LSD_{5\%}$=3.9). At a soil depth of 0–10 cm the soil moisture content varied between 9.2 and 15.4% (m/m), no significant differences were observed between the experiments. At 10–20 cm, where the soil moisture contents ranged from 11.5–16.6% (m/m), which represents an approximately 69% difference between the values obtained for experiments C and F. In the case of the 20–30 cm soil layer, 64% higher soil moisture values were recorded in experiment F than in experiment D. The lowest moisture content in experiment B, 11.8% (m/m), was measured at 30–40 cm. The highest moisture content for experiment E was recorded at 40–50 cm, which was 62% higher than in experiment B. The growth in soil penetration resistance may be a consequence of the low moisture content experienced at depths of 30–40 cm and 40–50 cm (30–40 cm=3.4 MPa; 40–50 cm=3.2 MPa). Beke (2006) also reported that in dry years higher penetration resistance values are generally obtained due to the lower moisture content. Rátonyi (1999) found that in the moisture range examined the penetration resistance grew in inverse proportion to the decrease in soil moisture content. In 2004 there was no significant difference between the individual experiments in the upper 0–10 cm layer. In 2005 (Fig. 7) significant differences were recorded

at 20–30 cm ($LSD_{5\%}$=1.7) and at 30–40 cm ($LSD_{5\%}$=1.9). The moisture content of the soil varied between 7.9 and 11.9% (m/m) at 20–30 cm, which represents more than 66% difference between the corresponding values of experiments D and A. For experiment D the soil moisture content at 30–40 cm was almost 73% lower than for experiment A. No significant differences were perceived between the experiments at other depths. In 2005 the soil moisture content at depths lower than 40 cm decreased in all the experiments. No significant differences in soil moisture were found between the experiments at any soil depths in 2006 (Fig. 8). The highest soil moisture contents were observed at a depth of 30–40 cm in all the experiments. As in 2005, the soil moisture content decreased in all the experiments at depths lower than 40 cm, with values of 10.8–14.8% (m/m), compared with 6.2–7.5% (m/m), in the upper 0–10 cm layer. Although the soil moisture values were not high at any depth, they were 51–57% lower at 40–50 cm than in the surface soil, so the difference is probably not due only to tillage operations performed at optimal moisture content. In 2006 compact layers that would hamper moisture transport were not detected at the depths examined, so there was nothing to prevent precipitation from penetrating the soil down to deeper layers. Based on the results of penetration resistance and soil moisture content measurements, it can be concluded that 2006 was the most favourable year from a crop production point of view.

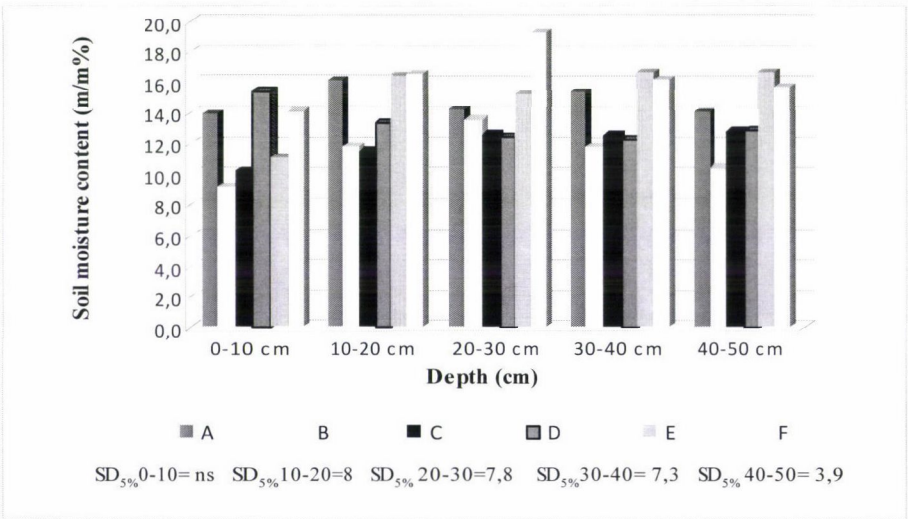

Fig. 6. Changes in soil moisture content at depths of 0–50 cm in 2004

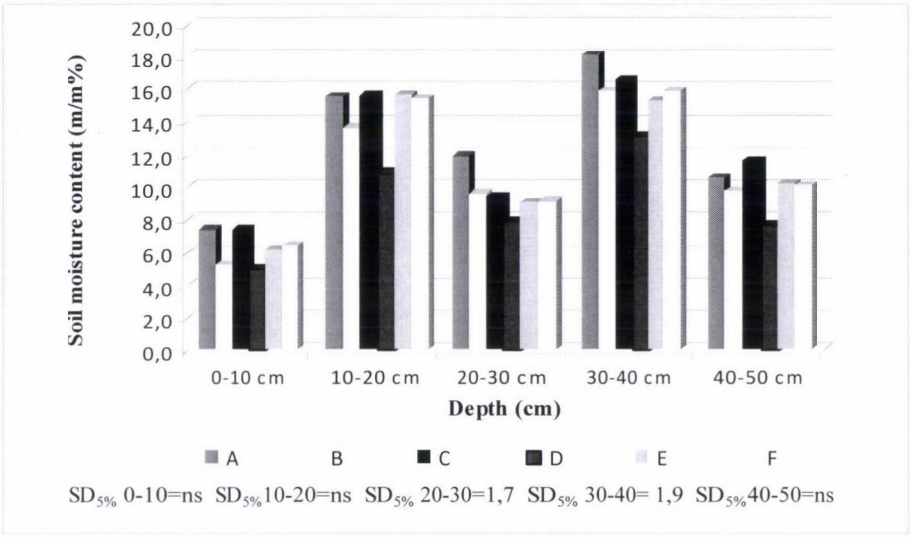

Fig. 7. Changes in soil moisture content at depths of 0–50 cm in 2005

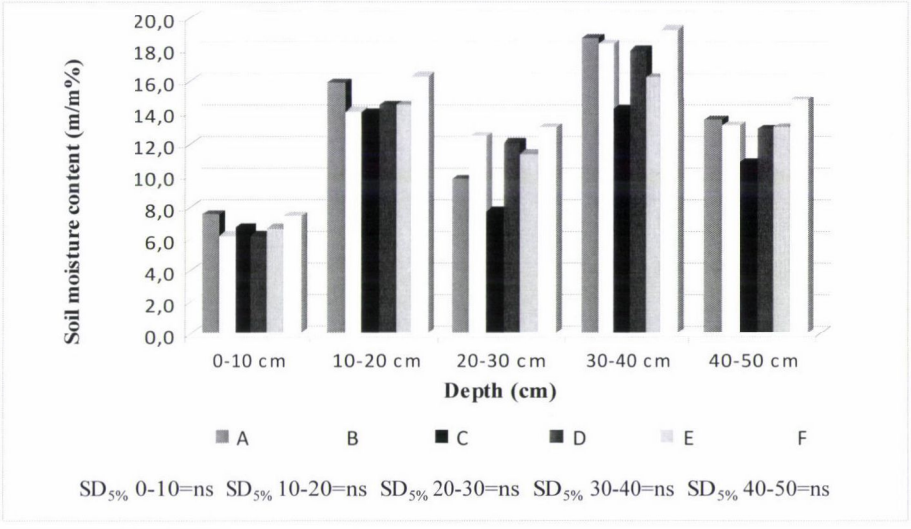

Fig. 8. Changes in soil moisture content at depths of 0–50 cm in 2006

Conclusions

In field experiments the effect of conventional tillage on the physical condition of the soil was assessed for three years on six separate farms by measuring penetration resistance and soil moisture content. It was concluded that in 2004 adversely compact layers were formed in three experiments below the depth of primary tillage. In 2005, when there was 20–23% more precipitation

than in the other two years, no adversely compacted layers were found at any depths in any of the experiments. The favourable PR values were attributed to tillage having been performed at the ideal moisture range and at optimal quality. Although significant differences in compaction were found at depths of 0–10 cm and 10–20 cm in the last year of the experiment, no adverse soil compaction was observed.

All in all it can be concluded that significant differences in PR were found between the six field experiments at tillage depth in the first year, below tillage depth in the second year, and in the upper 0–20 cm in the third. Soil moisture content was investigated simultaneously with the PR measurements for all the field experiments. In the first year of the survey experiment B had the lowest moisture content at all depths, with the exception of the 20–30 cm soil layer. The low moisture content measured at depths of 30–40 cm and 40–50 cm could have been responsible for the rise in PR. In 2005 significant differences were found between the experiments at depths of 20–30 cm and 30–40 cm. In 2006 no significant differences were found at any depth.

When examining the effects of traditional tillage, it was revealed that at no depths did adversely compact layers occur throughout the examined period, when tillage was performed at an identical depth for three years. The penetration of precipitation into deeper layers, which influences the effectiveness of crop production, was not hampered by adversely compact isolating layers. In addition, the physical properties of the soil may have helped to ensure optimum soil conditions.

References

Baráth, C., Ittzés, A., Ugrósdy, G. (1996): *Biometria.* (Biometry.) Mezőgazda Kiadó, Budapest.

Beke, D. (2006): *Talajtömörödés és nedvességtartalom vizsgálat szántóföldi tartamkísérletekben.* (Soil compaction and soil moisture studies in long-term field experiments.) PhD Thesis, Keszthely.

Birkás, M. (2000): *A talajtömörödés helyzete Magyarországon. Következményei és enyhítésének lehetőségei.* (Soil compaction occurrence and consequences in Hungary; Possibilities for alleviation.) DSc Thesis, Budapest.

Birkás, M., Gyuricza, C. (2004): A talajhasználat és a klimatikus hatások kapcsolata. (Relationship between soil use and climatic effects.) In: Birkás, M., Gyuricza, C. (eds.), *Talajhasználat – Műveléshatás – Talajnedvesség.* (Land use – Tillage effect – Soil moisture). Quality-Press Nyomda & Kiadó Kft., Budapest. pp. 10–43.

Braunack, M. V., McPhee, J. E., Reid, D. J. (1995): Controlled traffic to increase productivity of irrigated row crops in the semi-arid tropics. *Aust. J. Expl. Agric.*, **35**, 503–513.

Chan, K. Y., Oates, A., Swan, A. D., Hayes, R. C., Dear, B. S., Peoples, M. B, (2006): Agronomic consequences of tractor wheel compaction on a clay soil. *Soil Tillage Res.*, **89**, 13–21.

Daróczi, S., Lelkes, J. (1999): A szarvasi PENETRONIK talajvizsgáló nyomószonda alkalmazása. (Application of the PENETRONIK penetrometer developed in Szarvas.) *Gyakorlati Agroforum*, **10**(7), 16–18.

Ellington, A. (1986): Effects of deep ripping, direct drilling, gypsum and lime on soils, wheat growth and yield. *Soil Tillage Res.*, **8**, 29–49.

Gyuricza, C. (2001): A szántóföldi talajhasználat környezetvédelmi vonatkozásai. (Environment protection aspects of field soil use.) pp. 175–180. In: Gyuricza, C., Birkás, M., Percze, A., Schmidt, R., Vincze, M. (eds.), *A szántóföldi talajhasználat alapjai.* (Basis of arable land use.) Akaprint Nyomdaipari Kft., Budapest.

Gyuricza, C., Liebhard, P., Rosner, J. (2004): Talajökológiai tényezők vizsgálata talajművelési tartamkísérletben. (Study of soil ecological factors in long-term tillage experiments.) pp. 96–110. In: Birkás, M., Gyuricza, C. (eds.), *Talajhasználat – Műveléshatás – Talajnedvesség.* (Land use – Tillage effect – Soil moisture). Quality-Press Nyomda & Kiadó Kft., Budapest.

Hakansson, I. (1990): A method for characterizing the state of compactness of the plough layer. *Soil Tillage Res.*, **16**, 105–120.

Hamza, M. A., Anderson, W. K. (2003): Responses of soil properties and grain yields to deep ripping and gypsum application in a compacted loamy sand soil contrasted with a sandy clay loam soil in Western Australia. *Aust. J. Agric. Res.*, **54**, 273–282.

Hamza, M. A., Anderson, W. K. (2005): Soil compaction in cropping systems. A review of the nature, causes and possible solutions. *Soil Tillage Res.*, **82**, 121–145.

Kirkegaard, J. A., So, H. B., Troedson, R. J., Wallis, E. S. (1992): The effect of compaction on the growth of pigeonpea on clay soils. I. Mechanisms of crop response and seasonal effects on a Vertisol in a sub-humid environment. *Soil Tillage Res.*, **24**, 107–127.

McGarry, D. (1990): Soil compaction and cotton growth on a Vertisol. *Aust. J. Soil Res.*, **28**, 869–877.

McGarry, D., Chan, K. Y. (1984): Preliminary investigation of clay soil's behaviour under furrow irrigated cotton. *Aust. J. Soil Res.*, **22**, 99–108.

Passioura, J. B. (2002): Soil conditions and plant growth. *Plant Cell Environ.*, **25**, 311–318.

Radford, B. J., Bridge, B. J., Davis, R. J., McGarry, D., Pillai, U. P., Rickman, J. F., Walsh, P. A., Yule, D. F. (2000): Changes in the properties of a Vertisol and responses of wheat after compaction with harvester traffic. *Soil Tillage Res.*, **54**, 155–170.

Radford, B. J., Yule, D. F., McGarry, D., Playford, C. (2001): Crop response to applied soil compaction and to compaction repair treatments. *Soil Tillage Res.*, **61**, 157–166.

Rátonyi T. (1999): *A talaj fizikai állapotának penetrométeres vizsgálata talajművelési tartamkísérletben.* (Investigations on the physical condition of the soil in a long-term tillage experiment using a penetrometer.) PhD Thesis, Debrecen.

Sadras, V. O., O'Leary, G. J., Roget, D. K. (2005): Crop responses to compacted soil: capture and efficiency in the use of water and radiation. *Field Crops Res.*, **91**, 131–148.

Soane, B. D., van Ouwerkerk, C. (1994): Soil compaction problems in world agriculture. In: Soane, B. D., Van Ouwerkerk, C. (eds.), *Soil Compaction in Crop Production.* Elsevier Sci., Amsterdam, pp. 1–21.

Stefanovits, P. (1992): *Talajtan.* (Soil Science). Mezőgazda Kiadó, Budapest.

Sváb, J. (1981): *Biometriai módszerek a kutatásban.* (Biometrical Methods in Research Work.) Mezőgazdasági Kiadó, Budapest.

Virág, S. (2005): *A művelés hatása a talajok rögképződésére és a rögaprítás energetikai összefüggései.* (The effect of tillage on clod formation in soils and the energetical relations of clod chopping.) PhD Thesis, Debrecen.

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RESPONSE OF ONION (*Allium cepa* L.) YIELD TO WATER STRESS AND MINERAL BIOFERTILIZATION

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Two field experiments were conducted at the Experimental Station of the Faculty of Agriculture, Mansoura University, Egypt, during the 2006/2007 and 2007/2008 seasons. The results showed that a gradual increase in soil water tension from 24 centibars (cb) (5588.25 m^3/ha), i.e. 56.3% field capacity to 36 cb (2634.49 m^3/ha), i.e. 41.% field capacity, significantly decreased the average onion bulb weight (g), bulb diameter (cm), bulb length (cm), total bulb yield (t/ha) and marketable bulb yield (t/ha) and significantly reduced the total percentage loss after 2, 4 and 6 months in both seasons. Conversely, this increase in water tension significantly amplified the culled bulb yield (t/ha), bulb dry matter (%) and water use efficiency (kg/m^3). Normal water supplies (24 cb) clearly led to high percentage losses in bulb dry weight compared to the other water stress treatments. The results indicated that the application of 75% NPK plus the bio-fertilizer Soft Guard significantly improved the average bulb weight (g/plant), bulb diameter (cm), bulb length (cm), total bulb yield (t/ha), marketable bulb yield (t/ha) and dry matter (%) compared with the other fertilization treatments.

Key words: soil water tension, mineral and bio-fertilizers, water use efficiency

Introduction

Onion (*Allium cepa* L.) is one of the most important vegetable and field crops grown and used throughout the world and is grown under a wide range of climates from temperate to tropical. Soil water tension significantly affects both the bulb yield and the yield components. In this respect, Sorensen et al. (2002) reported that drought stress during the final growth stage forced the onions to mature, reducing the yield. The percentage of single-centre onion bulbs was lower when soil water stress occurred earlier in the growing season than when the stress occurred later. Woldetsadik (2003) reported that soil moisture stress at all growth stages severely affected the yield and quality of shallot (*Allium cepa* var. *ascolonicum*). Frequent irrigation at 25% depletion of available moisture

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throughout the growing season was required to achieve high yields. Mateen and Hassan (2005) concluded that 5 days was a better irrigation interval compared to other treatments in terms of plant growth and bulb yield. Maximum seedling survival percentages of 98% and 97% were observed in plots with a 5-day irrigation interval. Sprouting after harvest was significantly different after a 5-day irrigation interval than in other treatments. Amal et al. (2006) stated that the marketable bulb yield was decreased by 5.85%–14.19% with alternative furrow irrigation (AFI) at 30-day intervals, while a 14.77–21.19% increase was obtained with AFI every 15 days, compared with plants receiving water at every furrow irrigation. Kumar et al. (2007) showed that irrigation had a significant effect on the growth parameters of onion and subsequently influenced the bulb yield.

As onion has a high nitrogen demand, its productivity depends on the use of optimum fertilizer rates (Jayathilake et al. 2002; Tadav et al., 2005). Woldetsadik (2003) found that nitrogen fertilization promoted vegetative growth and exposed plants to soil moisture stress ahead of maturity, thus reducing the yield of rainfed shallots. When supplemental irrigation was provided, however, nitrogen fertilization in the range 75–150 kg/ha led to yield increases of about 10–15%. Katung et al. (2005) found that higher doses of nitrogen increased the marketable yield (10 t/ha) and significantly reduced losses after 5 months of storage, by 19.96% in 2002 and 37.12% in 2003. A significant response with increasing farmyard manure levels was recorded for onion yield and storage quality. El-Desuki et al. (2006) showed that the total bulb yield, marketable yield (exportable and local) and bulb quality were gradually and significantly increased by increasing the level of NPK fertilizers from 40 or 70 to 100% of the recommended dose of fertilization. Halvorson et al. (2006; 2007) reported that the total marketable fresh onion yield increased when the N rate was increased from 0 to 224 kg/ha. Balemi et al. (2007) found that the use of *Azotobacter* CBD-15 led to a saving of 50 kg N/ha without significantly affecting yield, with an average increase of 13.5% marketable yield in response to *Azotobacter* inoculation in the presence of 75 kg N/ha. Mahanthesh et al. (2008) reported that plants provided with *Azospirillum* + 100% NPK (125:50:125 NPK kg/ha) had better storage qualities under irrigated conditions during the rabi season.

The objectives of this study were to investigate the impact of soil water tension (irrigation water quantity) on the growth, yield and bulb quality of onion, to reduce the recommended NPK rate by 25% and replace it by biofertilizer, to elevate water use efficiency, i.e. to minimize irrigation water quantity without a marked decrease in onion bulb yield or quality, and to provide recommendations on how to reduce losses during the storage period.

Materials and methods

Field experiments were conducted at the Experimental Farm of the Faculty of Agriculture, Mansoura University, Egypt, during the 2006/2007 and 2007/2008 seasons. The objective of this investigation was to study the effect of soil water tension and combinations of mineral and bio-fertilizers on the yield and quality of onion, cv. Behairy Red, the seeds of which were obtained from the Onion Research Center, Giza, Egypt. The experiment was laid out in a completely

randomized block design with four replications. The soil of the experimental site was clayey, with pH values of 7.7 and 7.6 in the two seasons. Total nitrogen was 0.078 and 0.087%, available phosphate 8.9 and 9.32 ppm and available potassium 348.1 and 369.2 ppm, determined using the methodology of Page (1982).

Four irrigation treatments were applied, at 24 cb (irrigation on 5 occasions, totalling 5588.13 m^3/ha), i.e. 56.3% field capacity, 28 cb (4 irrigations totalling 4661.76 m^3/ha), i.e. 52.5% field capacity, 32 cb (3 irrigations totalling 3672.31. m^3/ha), i.e. 50.0% field capacity and 36 cb (2 irrigations totalling 2634.48 m^3/ha), i.e. 41.7% field capacity. Moisture percentages were calculated from a standard curve. Six fertilizer treatments, i.e. 100% NPK (240 kg N + 70 kg P_2O_5 + 60 kg K_2O)/ha, 75% NPK, 75% NPK + Alga 600, 75% NPK + Algreen, 75% NPK + Amino Total and 75% NPK + Soft Guard, were applied in a randomized complete block design with four replications. The biofertilizers (Amino Total, Soft Guard, Algreen and Alga 600) were made by the Technogreen Company (LEILI), Egypt, from soluble seaweed extracts.

The preceding crop was maize (*Zea mays* L.) in both seasons. Land preparation, transplanting, crop management, and all agronomic practices and treatments except the studied factors, were uniformly applied to plants in the nursery and permanent land, as normally done by farmers at the experimental location. Each basic experimental unit included 5 ridges, each 60 cm wide and 3 m long. The NPK fertilizers were applied as calcium superphosphate (15.5% P_2O_5), applied after dividing and before transplanting, ammonium sulphate (20.5% N) and potassium sulphate (48% K_2O), half of which was applied after transplanting immediately before irrigation, while the other half of the nitrogen and potassium was applied before the first irrigation for the 32 cb and 36 cb treatments and before the second irrigation for the 24 cb and 28 cb treatments. Transplanting took place during the first week of January, when seedlings were transplanted by hand on both sides of the ridges. The top portion of the transplants was cut back immediately before transplanting to reduce transpiration. The seedlings were irrigated immediately after transplanting, after which the aforementioned irrigation schedules were followed. Bio-fertilizers were applied as foliar spray 25, 40 and 55 days after transplanting, when most plots in the experiment were relatively wet. At harvest ten guarded plants were chosen at random from the outer ridges of each plot to determine bulb weight (g), bulb diameter (cm), bulb length (cm) and bulb dry matter (%). The total bulb yield, marketable bulb yield and culled bulb yield were determined in kg by harvesting the two middle rows per plot and then converted to t/ha. Water use efficiency (kg/m^3) was calculated according to Wright (1988) as:

$$\text{WUE} = \frac{\text{Total yield (kg/ha)}}{\text{Consumed water (m}^3\text{/ha)}}$$

The consumed water (m^3/ha) was estimated as:

Consumed water (m^3/ha) = (moisture percentage at field capacity – soil moisture percentage) × Root depth × Soil bulk density (1.76) × 10000

The total loss (%) was assessed after 2, 4 or 6 months of storage.

All the data were statistically analysed using analysis of variance (ANOVA) for a randomized complete block design for each experiment, then combined analysis was done between irrigation treatments (Gomez and Gomez, 1984). The new least significant differences described by Waller and Duncan (1969) were calculated between treatment means at the 5% level of probability. Statistical analysis was performed using SAS (1996) software.

Results and discussion

Effect of water stress

The results in Tables 1, 2 and 3 showed that increasing the soil water tension from 24 cb to 36 cb significantly decreased the mean onion bulb weight (g), bulb diameter (cm), bulb length (cm), total bulb yield (t/ha) and marketable

bulb yield (t/ha), and significantly reduced the total loss percentage after 2, 4 or 6 months, while significantly augmenting the culled bulb yield (t/ha), bulb dry matter (%) and water use efficiency in kg/m^3 with each increase in soil water tension. The highest values of onion bulb weight (80.8 and 79.7 g in the first and second seasons, respectively) were recorded for normal irrigation at 24 cb. The greatest bulb diameter (5.91 and 6.26 cm) and bulb length (5.41 and 5.34 cm) were also recorded for normal irrigation tension (24 cb) in the first and second seasons. Raising the soil water tension from 24 cb to 28 cb caused a decrease of 8.54% in total bulb yield and 9.87% in marketable yield over the two seasons. Furthermore, a rise in soil water tension from 32 cb to 36 cb significantly reduced the marketable onion yield by 12.49% over the two seasons. The maximum culled bulb yield was recorded at 36 cb, with values of 1.635 and 1.866 t/ha in the two seasons.

Irrigation at a soil water tension of 36 cb, significantly amplified the bulb dry matter by 16.32 and 16.21% in the first and second seasons, respectively, and the water use efficiency by 41%, 22.25% and 19.35% compared with irrigation at soil water tension of 24, 28 and 32 cb respectively, averaged over the two seasons. In addition, normal irrigation (24 cb) caused the greatest loss in bulb dry weight (8.06 and 9.22% in the two seasons), while irrigation at 36 cb gave the significantly lowest loss in bulb dry weight (5.43 and 5.12% in the two seasons). The highest average fresh bulb weight (g), bulb diameter (cm), bulb length (cm) and total bulb yield (t/ha) were recorded for the recommended soil water tension of 24 cb. The results indicated that at increasing soil water tension the soil moisture content was insufficient to supply the needs of the onion plants, resulting in reduced growth and the development of smaller bulbs.

Increasing the soil water tension from 24 to 36 cb greatly enhanced bulb dry matter (%), while more plentiful water supplies diluted the cell solution, therefore reducing the bulb dry matter percentage. Increasing the soil water tension from 24 cb to 36 cb also increased the water use efficiency (kg/m^3) of air-dried bulbs in both seasons (Fig. 1), though the difference between 28 cb and 32 cb was not significant in the first season. The crops extracted more water from the soil in the drought treatments (32 and 36 cb) than when there were abundant supplies of irrigation water (24 and 28 cb). Koriem et al. (1999) concluded that the total bulb yield was significantly amplified by increasing soil moisture up to 30% depletion of available soil moisture. They added that water consumption increased with an increase in available soil moisture. Mohamed and Gamie (2000) reported that total soluble solids declined with an increase in available soil moisture.

Table 1
Average bulb weight (g), bulb diameter (cm), bulb length (cm) and total bulb yield (t/ha) as affected by water tension and by mineral and bio-fertilization in two seasons

Treatments	Bulb weight		Bulb diameter		Bulb length		Total bulb yield	
	2006	2007	2006	2007	2006	2007	2006	2007
Irrigation tension:								
24 cb	80.8	79.7	5.91	6.26	5.41	5.34	40.067	37.732
28 cb	73.9	76.1	5.40	5.84	5.12	5.24	36.807	36.176
32 cb	66.7	62.1	4.98	5.21	4.92	4.95	29.317	30.347
36 cb	60.0	53.3	4.70	4.45	4.66	4.43	26.896	26.154
F test	**	**	**	**	**	**	**	**
NLSD (5%)	2.1	1.1	0.12	0.07	0.16	0.07	0.354	0.24
NLSD (1%)	2.9	1.5	0.16	0.10	0.23	0.10	0.484	0.331
Fertilization:								
100% NPK + Water	71.4	70.2	5.21	5.58	5.22	5.04	34.548	33.175
75% NPK + Water	63.1	59.6	4.93	4.96	4.75	4.64	28.382	28.779
75% NPK + Alga 600 (1000 ppm)	72.7	68.4	5.35	5.39	5.01	4.88	34.167	32.754
75% NPK + Algreen (3000 ppm)	70.9	67.2	5.21	5.26	5.01	5.13	33.970	32.187
75% NPK + Amino Total (1000 ppm	71.6	70.1	5.39	5.68	5.16	5.11	35.714	34.093
75% NPK + Soft Guard (4000 ppm)	72.3	71.4	5.40	5.78	5.01	5.14	35.852	34.629
F test	**	**	**	**	**	**	**	**
NLSD (5%)	2.4	1.2	0.14	0.09	0.19	0.12	0.535	0.23
NLSD (1%)	3.2	1.6	0.19	0.12	0.26	0.16	0.707	0.30
Interaction F test	NS	NS	NS	NS	NS	NS	NS	NS

NLSD: New least significant differences; NS: non-significant; **: Significant at the P = 0.01 level

Table 2
Average marketable bulb yield (t/ha), culled bulb yield (t/ha), bulb dry matter (%) and water use efficiency (kg/m^3) as affected by water tension and by mineral and bio-fertilization in two seasons

Treatments	Marketable bulb yield		Culled bulb yield		Bulb dry matter		Water use efficiency	
	2006	2007	2006	2007	2006	2007	2006	2007
Irrigation tension:								
24 cb	40.674	36.357	1.392	1.376	14.16	13.43	7.59	6.81
28 cb	35.355	34.698	1.454	1.478	14.79	14.59	7.96	7.83
32 cb	27.820	28.803	1.497	1.545	15.27	15.22	8.05	8.33
36 cb	25.261	24.288	1.635	1.866	16.32	16.21	10.30	10.01
F test	**	**	**	**	**	**	**	**
NLSD (5%)	0.348	0.230	0.038	0.041	0.25	0.13	0.23	0.13
NLSD (1%)	0.476	0.314	0.054	0.059	0.34	0.18	0.33	0.19
Fertilization:								
100% NPK + Water	33.077	31.635	1.471	1.540	14.75	14.32	8.65	8.39
75% NPK + Water	26.939	27.182	1.442	1.595	15.28	15.37	7.51	7.32
75% NPK + Alga 600 (1000 ppm)	32.613	31.157	1.554	1.597	15.29	14.87	8.61	8.21
75% NPK + Algreen (3000 ppm)	32.463	30.719	1.507	1.468	15.17	14.71	8.53	8.12
75% NPK + Amino Total (1000 ppm)	34.208	32.466	1.504	1.628	15.04	14.80	8.95	8.64
75% NPK + Soft Guard (4000 ppm)	34.362	33.058	1.488	1.571	15.28	15.10	8.94	8.76
F test	**	**	NS	NS	NS	**	**	**
NLSD (5%)	0.535	0.239	–	–	–	0.13	0.35	0.13
NLSD (1%)	0.707	0.315	–	–	–	0.16	0.48	0.18
Interaction F test	NS	NS	NS	NS	NS	NS	NS	NS

NLSD: New least significant differences; NS: non-significant; **: Significant at the P = 0.01 level

Table 3
Average losses (%) assessed after 2, 4 and 6 months and total loss (%) after 6 months, as affected by water tension and by mineral and bio-fertilization in two seasons

Treatments	After 2 months		After 4 months		After 6 months		Total loss after 6 months	
	2006	2007	2006	2008	2006	2007	2006	2007
Irrigation tension:								
24 cb	2.57	42.9	2.62	2.93	2.87	3.33	8.06	9.22
28 cb	2.47	42.5	2.10	2.22	2.67	2.89	7.25	7.66
32 cb	2.22	1.98	1.92	1.77	2.56	2.14	6.72	5.92
36 cb	1.70	1.60	1.59	1.43	2.12	2.12	5.43	5.12
F test	**	**	**	**	**	**	**	**
NLSD (5%)	0.23	0.21	0.230	0.19	0.24	70.1	0.48	0.41
NLSD (1%)	0.32	0.29	0.32	0.26	0.34	0.23	0.67	0.57
Fertilization:								
100% NPK + Water	2.64	22.7	92.3	72.5	2.64	23.0	7.66	8.29
75% NPK + Water	2.41	92.2	2.00	1.83	2.64	32.4	7.07	6.56
75% NPK + Alga 600 (1000 ppm)	2.11	92.0	2.17	2.13	2.52	2.63	6.80	6.87
75% NPK + Algreen (3000 ppm)	2.16	2.13	2.04	2.09	2.54	2.72	6.76	6.96
75% NPK + Amino Total (1000 ppm)	2.08	2.19	1.93	2.08	2.38	2.68	6.41	6.95
75% NPK + Soft Guard (4000 ppm)	2.06	2.18	1.80	1.83	2.62	2.24	6.50	6.25
F test	**	**	**	**	NS	**	**	**
NLSD (5%)	0.22	10.2	0.20	0.15	–	0.19	0.42	50.3
NLSD (1%)	0.30	0.28	0.27	0.21	–	0.25	0.57	0.47
Interaction F test	NS	NS	*	*	NS	NS	**	**

NLSD: New least significant differences; NS: non-significant; *, **: Significant at the P = 0.1 and 0.01 levels, respectively

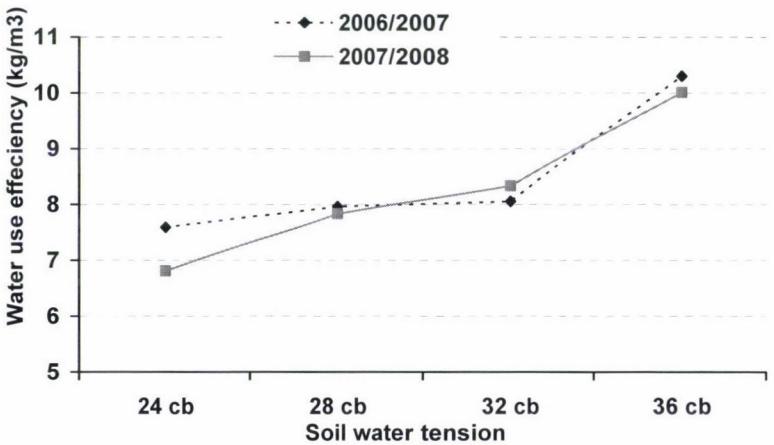

Fig. 1. Water use efficiency in kg/m^3 as affected by soil water tension in the 2006/2007 and 2007/2008 seasons

Effect of mineral and bio-fertilization

The results in Tables 1, 2 and 3 showed that the replacement of 75% recommended NPK fertilizers (180 kg N + 53 kg P_2O_5 + 45 kg K_2O/ha) by foliar spraying with Soft Guard at 4000 ppm or Amino Total at 1000 ppm as bio-fertilizer significantly increased the average bulb weight (g), bulb diameter (cm), bulb length (cm), total bulb yield (t/ha), marketable bulb yield (t/ha), bulb dry matter (%) and water use efficiency (kg/m^3) as compared with the application of 100% NPK or 75% NPK fertilizer + water. There were no significant differences between the latter in either season. In both seasons the lowest total bulb yield loss after 2, 4 or 6 months of storage was recorded when using 75% NPK + Soft Guard or Amino Total. Maximum bulb weight was achieved in the same treatment, which also resulted in the significantly largest bulb diameter. The combination of 75% NPK plus Amino Total or Soft Guard also gave the highest values of total bulb yield (35.714 and 34.094, and 35.852 and 34.629 t/ha, respectively, in the two seasons) and marketable bulb yield (34.208 and 32.466, and 34.362 and 33.058 t/ha, respectively) in the two seasons. In addition, the results indicated that the culled bulb yield was not significantly affected by the mineral and biofertilization treatments in either season. Fertilization with 75% NPK + Amino Total or Soft Guard significantly amplified the water use efficiency by 22.34 and 3.9 % compared with 75% NPK + water and 100% NPK averaged over the two seasons (Fig. 2). The results indicated that fertilization using a combination of 75% NPK and Amino Total or Soft Guard gave significant increases in bulb weight, bulb diameter, total bulb yield and marketable bulb yield in both seasons. The application of 100% NPK (240 kg N + 70 kg P_2O_5 + 60 kg K_2O/ha) significantly increased the marketable yield by 16.36% as compared with 75% NPK. The application of 75% NPK + Alga 600 or Algreen (seaweed extract) led to a 5.32% reduction in marketable bulb yield compared with 75% NPK plus Amino total or Soft Guard in both seasons (Table 3). The application of 100% NPK or 75% NPK plus Soft Guard or Amino Total resulted in the greatest bulb length, with no significant difference between the treatments in either season. The increase in total bulb yield due to the addition of 75% NPK with Soft Guard or Amino Total may be attributed to the role of nitrogen in increasing the meristematic activity of onion tissues and thus the internal length of the bulbs, leading to a higher marketable bulb yield.

Moreover, the addition of P and K together with nitrogen may increase the quantity of metabolites synthesized in the plant, which may then be translocated to the bulb (Sorensen et. al., 2002). Balemi et al. (2007) concluded that the highest marketable bulb yield could be produced by applying 75 kg N in combination with *Azotobacter* as biofertilizer. The role of Soft Guard in improving WUE might be due to an increase in the resistance of onion plants to drought. It may also enhance onion growth and improve productivity. Amino Total, on the other hand, helps to increase the chlorophyll concentration and improve the absorption of macro and trace elements. The N, P and K fertilizers also stimulate the growth and development of onion plants.

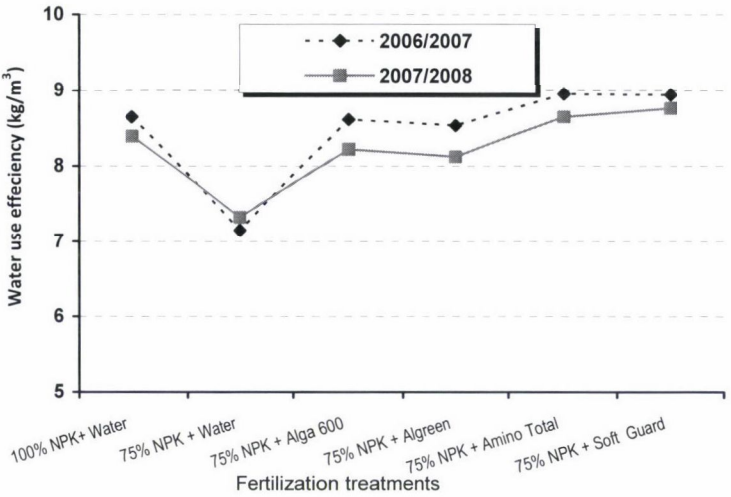

Fig. 2. Water use efficiency in kg/m^3 as affected by mineral and bio-fertilization treatments in 2006/2007 and 2007/2008 seasons

Interaction effect

The significant interaction between the irrigation treatments and the mineral bio-fertilizers influenced total post-harvest losses after 4 and 6 months, as shown in Tables 4 and 5. The highest losses in dry bulb weight after 4 months were recorded for irrigation at a soil water tension of 24 cb combined with fertilization with 100% NPK in both seasons (3.43 and 3.83%), (respectively). The same treatment resulted in the highest losses after 6 months (10.17 and 11.65% in the first and second seasons, respectively). The lowest losses after 4 months (1.38 and 1.25%) and 6 months (5.09 and 4.53%) were obtained with irrigation at 36 cb and 75% NPK + Soft Guard in the two seasons.

Table 4

Mean losses in dry bulb weight assessed after 4 months as affected by the interaction between soil water tension and mineral and bio-fertilizers in the 2006/2007 and 2007/2008 seasons

Treatments	2006/2007				2007/2008			
Irrigation	24 cb	28 cb	32 cb	36 cb	24 cb	28 cb	32 cb	36 cb
100% NPK + Water	3.43	2.43	1.88	1.83	3.83	2.75	2.03	1.68
75% NPK + Water	2.78	2.00	1.80	1.43	2.73	1.73	1.63	1.28
75% NPK + Alga	2.85	1.83	2.35	1.65	3.00	2.13	2.00	1.38
75% NPK + Algreen	2.35	2.38	1.85	1.60	2.58	2.50	1.73	1.58
75% NPK + Amino Total	2.15	2.15	1.78	1.65	2.70	2.30	1.88	1.43
75% NPK + Soft Guard	2.15	1.83	1.85	1.38	2.78	1.90	1.38	1.25
F-test			*				*	
NLSD 5%			0.49				0.47	

NLSD: New least significant differences; NS: non-significant; * Significant at the P = 0.1 level

Table 5

Mean total losses in dry bulb weight assessed after 6 months as affected by the interaction between soil water tension and mineral and bio-fertilizers in the 2006/2007 and 2007/2008 seasons

	Losses assessed after 6 months %							
Treatments	2006/2007				2007/2008			
Irrigation	24 cb	28 cb	32 cb	36 cb	24 cb	28 cb	32 cb	36 cb
100% NPK + Water	10.17	8.23	6.18	6.04	11.65	9.08	6.38	6.08
75% NPK + Water	8.67	7.30	6.88	5.41	9.08	6.85	5.60	4.73
75% NPK + Alga	8.33	6.25	7.12	5.51	9.28	7.35	6.10	4.75
75% NPK + Algreen	7.47	7.14	7.08	5.35	8.20	7.90	6.08	5.65
75% NPK + Amino total	6.81	7.31	6.31	5.21	8.68	7.53	6.60	5.00
75% NPK + Soft Guard	6.92	7.27	6.74	5.09	8.43	7.28	4.78	4.53
F-test			**				**	
NLSD 5%			0.97				0.89	
NLSD 1%			1.34				1.23	

NLSD: New least significant differences; NS: non-significant; **: Significant at the P = 0.01 level

Conclusions

The results clearly indicated that the greatest marketable bulb yield and the lowest total losses in dry bulb weight after storage were obtained at a soil water tension of 24 cb with 75% NPK plus spraying with 1000 ppm Amino Total or 4000 ppm Soft Guard at 25, 40 and 55 days after transplanting. Irrigation at a soil water tension of 36 cb, however, increased the water use efficiency by 41.11% and reduced the total loss in dry bulb weight after six months by 39.12% in the same fertilizer treatment compared with irrigation at a soil water tension of 24 cb.

References

Amal, F. E., Mostafa, A. K., Abd El-Maksoud, H. H. (2006): Effect of alternate furrow irrigation and transplanting distance on water utilization efficiency for onion crop. *Misr. J. Agric. Eng.*, **23**, 137–150.

Balemi, T., Pal, N., Saxena, A. K. (2007): Response of onion (*Allium cepa* L.) to combined application of biological and chemical nitrogenous fertilizers. *Acta Agric. Slovenia*, **89**, 107–114.

El-Desuki, M., Asmaa, R. M., Magda, M. H. (2006): Response of onion plants to minerals and bio-fertilizers application. *Res. J. Agric. Biol. Sci.*, **2**, 292–298.

Gomez, K. A., Gomez, A. A. (1984): *Statistical Procedures for Agricultural Research.* 2nd ed. Wiley Inter Science Publ., New York. pp. 357–423.

Halvorson, A. D., Bartolo, M., Reule, C. A., Barrada, A. (2006): Onion response to nitrogen fertilization under drip and furrow irrigation. *Proc. Natl. Allium Conf.*, College Station. TX, USA. pp. 73–78.

Halvorson, A. D., Bartolo, M. E., Reule, C. A., Barrada, A. (2007): Nitrogen effects on onion yield under drip and furrow irrigation. *Agron. J.*, **100**, 1062–1069.

Jayathilake, P. K. S., Reddy, I. P., Srihari, D., Neeraja, G., Ravinder, R. (2002): Effect of nutrient management on growth, yield and yield attributes of Rabi onion (*Allium cepa* L.). *Veg. Sci.*, **29**, 184–185.

Katung, M. D., Hassbini, I. M., Olarewaju, J. D. (2005): Yield and storability of onion (*Allium cepa* L.) as influenced by organic and inorganic fertilizers in the Sudan Savanna region of Nigeria. *Nig. J. Hort. Sci.,* **10**, 82–86.

Koriem, S. O., El-Koliey, M. M., El-Sheek, H. M. (1999): Effect of drought conditions on yield quality and some water relationships of onion. *Assuit J. Agric. Sci.*, **30**, 75–84.

Kumar, S., Imtiyaz, M., Ashwani, K., Rajbir, S. (2007): Response of onion (*Allium cepa* L.) to different levels of irrigation water. *Agric. Water Management*, **89**, 161–166.

Mahanthesh, B., Sajjan, M. R. P., Srinivasa, V., Harshavardhan, M., Thippesha, D. (2008): Studies on the influence of bio-fertilizers with levels of NPK on the yield and processing qualities of onion (*Allium cepa* L.) cv. Bellary Red in rabi season under irrigated situation. *Res. Crops*, **9**, 98–102.

Mateen, U. L., Hassan, K. (2005): Effect of irrigation intervals on growth and yield of onion varieties Swat–1 and Phulkara. *J. Appl. Sci. Res.*, **1**, 112–116.

Mohamed, K. A., Gamie, A. A. (2000): Effect of irrigation regimes on yield and some bulb quality characters of onion varieties. *Assiut J. Agric. Sci.*, **31**, 115–127.

Page, A. L. (1982): *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*. Amer. Soc. Agron., Madison, WI, USA.

SAS (1996): *SAS/STAT User's Guide: Statistics*. Version 7. SAS Institute Inc., Cary, NC, USA.

Sorensen, J. N., Grevsen, K., Booij, R., Neeteson, J. (2002): Nitrogen and water stress affects sprouting in bulb onions stored over winter. *Acta Hort.*, **571**, 79–86.

Tadav, B. D., Khandelwal, R. B., Sharma, Y. K. (2005): Use of bio-fertilizer (*Azospirillum*) in onion. *Indian Hort.*, **62**, 168–170.

Waller, R. A., Duncan, B. D. (1969): A Bayes rule for the symmetric multiple comparison problem. *J. Amer. Stat. Assoc.,* **64**, 1484–1503.

Woldetsadik, K. (2003): *Shallot (*Allium cepa *var.* ascolonicum*) responses to plant nutrients and soil moisture in a sub-humid tropical climate*. PhD Thesis, Dept. Crop Sci., Swedish Univ. Agric. Sci., ISBN 91-576-6195-2.

Wright, G. W. (1988): Daily and seasonal evapotranspiration and yield of irrigated alfalfa in southern Idaho. *Agron. J.,* **80**, 662–669.

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MYCELIAL COMPATIBILITY OF HUNGARIAN *Macrophomina phaseolina* ISOLATES

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The charcoal root disease caused by *Macrophomina phaseolina* (Tassi) Goidanich leads to considerable damage in hot, dry seasons in many parts of the world, including Hungary. The present study investigated the mycelial compatibility of 53 *Macrophomina phaseolina* isolates, collected from sunflower, maize and soybean in different regions of Hungary, in order to characterize the diversity of the pathogen. Compatible isolates were identified by growing the isolates in direct contact with each other on potato-dextrose agar medium under laboratory conditions. Most isolates were compatible. Only 24 pairs of all the possible paired combinations showed incompatible relationships. Even geographically distant isolates were found to be compatible. Some Serbian isolates were compatible with all the Hungarian isolates and one Spanish isolate tested in this work. The latter exhibited incompatibility only with a single Hungarian isolate. These results suggest that the same or very similar genotypes may spread over long distances, probably through the transportation of seeds or crops contaminated with microsclerotia. This is the first report on the compatibility of *Macrophomina phaseolina* isolates in Hungary.

Keywords: *Macrophomina phaseolina*, compatibility, incompatible relationship

Introduction

Charcoal rot disease, caused by *Macrophomina* (=*M.*) *phaseolina* (Tassi) Goidanich [synanamorph: *Rhizoctonia bataticola* (Taubenhaus) E.J. Butler], is an economically important problem in a number of crops. The disease can be diagnosed on the basis of the symptoms: ash grey spots on the stems and small, black microsclerotia in the pith and root tissues. *M. phaseolina* is a soil- and seed-borne generalist fungal pathogen that has a global distribution and infects more than 700 dicotyledonous and monocotyledonous plant species worldwide (Purkayastha et al., 2006). In Hungary it causes serious damage on sunflower, maize, legumes, paprika and many other crops, especially in dry, hot seasons

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(Békési et al., 1970; Kadlicskó, 1993). The pathogen was also identified in Hungary on tree species such as apricot and blue spruce (Vajna and Rozsnyai, 1995; Fischl et al., 2008). In recent years the most serious infections with *Macrophomina* have been observed on sunflower. Békési (2002) reported 90% infection in sunflower in 2002, resulting in a 30–35% loss of yield. In 2007 *M. phaseolina* was by far the most important pathogen on sunflower in Hungary (Békési, 2010). In 2008 the damage caused by *M. phaseolina* was manifested as a 10% loss of yield (Békési, 2008). It is therefore essential to study the biology and diversity of the pathogen and the factors affecting the level of damage.

The variability of the pathogen in a geographical area can be described using mycelium compatibility tests. Two isolates are compatible if their hyphae produce anastomoses (hypha bridges). Genetic recombination may occur through the exchange of nuclei during the migration of nuclear elements from one isolate to another through the anastomoses (Mihail and Taylor, 1995). This process, known as parasexual recombination, is typical of species that reproduce mainly or exclusively in an asexual manner or are homothallic. In nature, when two isolates are present at the same place at the same time, the creation of heterocaryons is possible. In the case of incompatible mycelia, anastomoses cannot be formed between hyphae of different isolates. Blocking zones (barriers) are often created, and mycelia that come into contact with each other may perish (Zándoki et al., 2005). Mihail and Taylor (1995) found that *M. phaseolina* isolates from divergent geographical locations readily underwent hyphal fusion when paired together, implying that there were no barriers to this form of genetic exchange.

The compatibility of *M. phaseolina* isolates from Hungary is unknown. The aim of the present study was to investigate this pattern in isolates collected in different Hungarian regions and to describe the diversity of the pathogen.

Materials and methods

Macrophomina phaseolina isolates were collected from 39 sites in Hungary (Fig. 1) from sunflower, maize and soybean. Scrapings from the infected plant debris were placed on potato dextrose agar (PDA) medium. Pure cultures were made by repeated passage. An isolate from Spain and one from Serbia were collected from sunflower, and another Serbian isolate from sugarbeet. The origin and hosts of the isolates are shown in Table 1. To study the compatibility of 53 isolates included in this work, all the isolates were grown in pairs in direct contact with each other on PDA medium under laboratory conditions. Agar blocks 5 mm in diameter were cut from the side of one-week-old pure cultures with microsclerotia and transferred to 9 cm Petri dishes containing 10 ml PDA medium. Three isolates were grown in each Petri dish, with three replications in each case and the dishes were placed in darkness in thermostats adjusted to 25°C. The type of compatibility between the isolates was determined 7 days after inoculation. The relationship was defined as compatible when the mycelia grew into each other in such a way that no boundaries could be seen and one continuous colony was created. The relationship was considered as incompatible when a clearly defined boundary (blocking zone) was formed between the colonies and some of the hyphae were destroyed. Inoculations were done in all possible paired combinations of the 53 isolates studied.

Table 1
Designations, locations of origin, sources, years of collection and host plants of the *M. phaseolina* isolates studied

Code of isolates	Origin	Source	Time of collection	Host
Mp 1	Balatonújlak (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 2	Bize (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 3	Boda (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 4	Bóly (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 5	Böhönye (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 6	Cserkeszőlő (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 7	Cserkeszőlő (Hungary)	Dr. Izabella Csöndes	2005	volunteer sunflower
Mp 8	Debrecen (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 9	Dunaföldvár (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 10	Gyulafirátót (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 11	Hódmezővásárhely (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 12	Kadarkút (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 13	Kaposvár-Toponár (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 14	Karcag (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 15	Kecskemét (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 16	Keszthely (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 17	Kéthely (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 18	Kunszentmárton (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 19	Lakitelek (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 20	Lepsény (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 21	Mesztegnyő (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 22	Nagykanizsa (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 23	Nyíregyháza (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 24	Pogányszentpéter (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 25	Röjtökmuzsaj (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 26	Sármellék (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 27	Szederkény (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 28	Szentes (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 29	Székkutas (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 30	Szigetvár (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 31	Tiszakürt (Hungary)	Dr. Izabella Csöndes	2005	sunflower
Mp 32	Tordas (Hungary)	Dr. László Gergely	2005	sunflower
Mp 33	Bóly (Hungary)	Dr. Izabella Csöndes	2005	soybean
Mp 34	Iregszemcse (Hungary)	Dr. Izabella Csöndes	2005	soybean
Mp 35	Keszthely (Hungary)	Dr. Izabella Csöndes	2005	soybean
Mp 36	Böhönye (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 37	Dombóvár (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 38	Kaposvár-Toponár (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 39	Kéthely (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 40	Marcali (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 41	Nagyrécse (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 42	Sármellék (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 43	Zalaapáti (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 44	Zalaszabar (Hungary)	Dr. Izabella Csöndes	2006	sunflower
Mp 45	Cordoba (Spain)	Dr. Leire Molinero-Ruiz	2006	sunflower
Mp 46	Sangaj (Serbia)	Dr. Vera Stojsin and	2004	sunflower
Mp 47	Rimski Sancevi (Serbia)	Dr. Ferenc Bagi	2003	sugarbeet
Mp 48	Keszthely (Hungary)	Dr. Sándor Kadlicskó	2002	soybean
Mp 49	Bicsérd (Hungary)	Ilona Walcz	1984	sunflower
Mp 50	Bicsérd (Hungary)	Ilona Walcz	1986	soybean
Mp 51	Bicsérd (Hungary)	Ilona Walcz	1999	maize
Mp 52	Bicsérd (Hungary)	Ilona Walcz	2004	sunflower
Mp 53	Szekszárd (Hungary)	Ilona Walcz	1982	sunflower

Fig. 1. Locations of origin of the *Macrophomina phaseolina* isolates included in this work

Results

It is typical of *M. phaseolina* that mycelia start to grow first, followed by the formation of microsclerotia, with which the fungus reproduces asexually. The hyphae of *M. phaseolina* branch at right angles (Fig. 2). *M. phaseolina* forms small, black, hard, smooth-surfaced microsclerotia (Fig. 3) which can be found in the root, stem and leaf tissues (Fig. 4A and B). Pycnidia of this fungus were only detected once in Hungary on the stem of bean plants (Vajna and Békési, personal communication). Unfortunately, the way in which this pathogen reproduces in Hungary is not well documented.

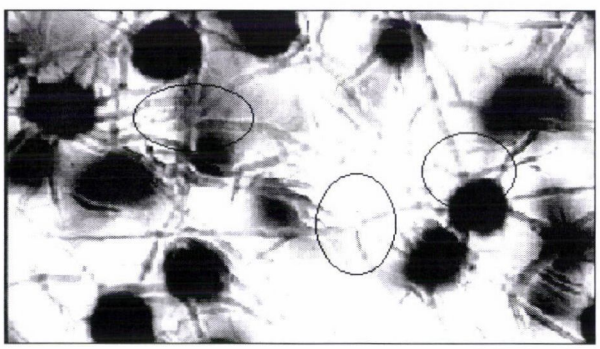

Fig. 2. Hyphae of *M. phaseolina* branching at right angles

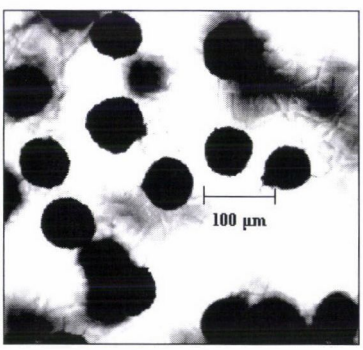

Fig. 3. Microsclerotia of *M. phaseolina*

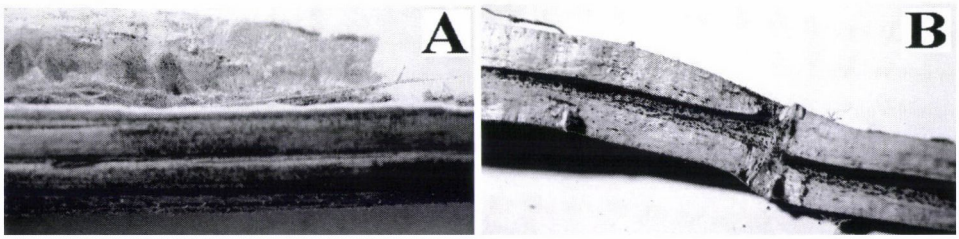

Fig. 4. A: Black microsclerotia in the pith of sunflower infected by *M. phaseolina*; B: microsclerotia of *M. phaseolina* in the stem of soybean

The type of compatibility between the isolates could be clearly defined on the 7th day after inoculation. One week after inoculation, colonies of compatible isolates grew into each other, blocking zones were formed between the colonies of incompatible isolates and the mycelia were thinned out. In the course of the examination, when the isolates were grown in all possible paired combinations, the isolates were mostly found to be compatible (Fig. 5). Table 2 shows the pairs of incompatible isolates. Only 24 of the 1378 combinations were incompatible. When the trials were repeated, similar results were obtained. Isolate Mp 34 showed the highest number of incompatible relationships with other isolates (Fig. 6).

It was surprising to find incompatibility between isolates from habitats situated close to each other, such as the pairs Mp 2–Mp 40, Mp 15–Mp 31, Mp 34–Mp 40, Mp 42–Mp 43 and Mp 42–Mp 44. This suggested that different genotypes may be present at the same time in regions close to each other.

The Serbian isolates were compatible with all the Hungarian isolates and also with the isolate from Spain, which only showed incompatibility with a single Hungarian isolate, Mp 34. Thus, geographically distant isolates were in general compatible to each other.

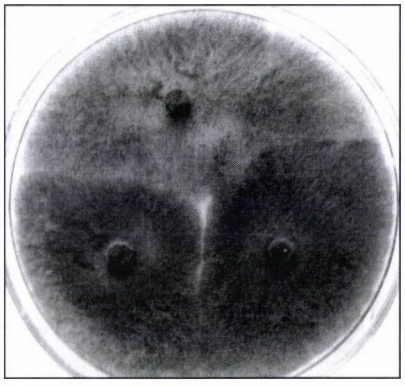

Fig. 5. Compatible relationship between Mp 43 (top), Mp 44 (bottom left) and Mp 49 (bottom right)

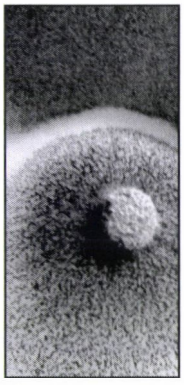

Fig. 6. Incompatible relationship between Mp 23 (top) and Mp 34 (bottom) isolates of *M. phaseolina*

Table 2
Incompatible *M. phaseolina* isolates

Incompatible pairs of isolates			
Mp 2–Mp 40	Mp 28–Mp 14	Mp 34–Mp 23 (Fig. 6)	Mp 34–Mp 44
Mp 3–Mp 19	Mp 29–Mp 8	Mp 34–Mp 24	Mp 34–Mp 45
Mp 5–Mp 10	Mp 29–Mp 22	Mp 34–Mp 33	Mp 38–Mp 6
Mp 25–Mp 1	Mp 31–Mp 7	Mp 34–Mp 40	Mp 38–Mp 11
Mp 25–Mp 31	Mp 31–Mp 15	Mp 34–Mp 42	Mp 42–Mp 43
Mp 28–Mp 12	Mp 31–Mp 30	Mp 34–Mp 43	Mp 42–Mp 44

Discussion

Studies on the compatibility of fungal isolates can be used to describe the diversity of certain species. When all possible paired combinations of *M. phaseolina* isolates were inoculated in direct contact with each other, most of the isolates were found to be compatible.

In Hungary mycelial compatibility was most recently investigated in *Sclerotinia sclerotiorum* by Zándoki et al. (2005). According to their work, *S. sclerotiorum* isolates from the same sites were often incompatible, while very distant isolates showed compatibility. In the present work the isolates from Serbia and Spain only showed incompatibility in one case. Similar to the work on *S. sclerotiorum* (Zándoki et al., 2005), geographically distant *M. phaseolina* isolates were found to be compatible. This proves that the same or similar genotypes may spread over long distances, probably with the transport of seeds and/or crops contaminated with microsclerotia.

Of all the possible paired combinations, only 24 pairs of isolates exhibited incompatibility. This is an important result, because the compatibility of the mycelia is a precondition for DNA exchange through anastomoses between two compatible isolates, thus raising the possibility of parasexual recombination and the consequent maintenance of genetic diversity in *Macrophomina*. Mihail and Taylor (1995) found that *Macrophomina* isolates from divergent geographical locations readily underwent hyphal fusion when paired together, implying that there were no barriers to this form of genetic exchange. The fact that Hungarian isolates are compatible with those from Serbia suggest that identical or similar genotypes may spread over long distances.

Further research is needed to investigate what factors influence mycelial compatibility in *M. phaseolina*, how the frequency of the genotypes changes in different habitats, and also to confirm the migration of DNA from one population to another through anastomoses.

References

Békési, P. (2002): A napraforgó legfontosabb betegségei és az ellenük alkalmazható védekezés lehetőségei. (The most important diseases of sunflower and potential protection against them.) *Gyakorlati Agrofórum,* **13**(1), 23–26.

Békési, P. (2008): A napraforgó állományok 2008. évi kórtani állapotának jellemzése. (Diseases in sunflower fields in 2008.) *Agrofórum,* **19**(11), 15–18.

Békési, P. (2010): A napraforgó betegségei és az agrotechnika. (Sunflower diseases and cultural operations.) *Agrofórum,* **21**(3), 38–40.

Békési, P., Vörös, J., Calvert, O. H. (1970): *Macrophomina phaseoli* in Hungary damaging sunflower. *Plant Dis. Rep.,* **54**, 286–287.

Fischl, G., Csöndes, I., Kadlicskó, S., Józsa, A. (2008): Az ezüstfenyő (*Pinus pungens* Engelm.) vörösödését és pusztulását okozó tényezők vizsgálata. [Study on the factors provoking the reddening and decline of blue spruce (*Picea pungens* Engelm.).] *Növényvédelem,* **44**, 401–402.

Kadlicskó, S. (1993): *A* Macrophomina phaseolina *(Tassi) Goid. előfordulása Magyarországon és a fontosabb gazda–parazita kapcsolatok vizsgálata.* [Occurrence of *Macrophomina phaseolina* (Tassi) Goid. in Hungary, and the investigation of important host–parasite relationships.] PhD Thesis, University of Pannonia.

Mihail, J. D., Taylor, S. J. (1995): Interpreting variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production, and chlorate utilization. *Can. J. Bot.*, **73**, 1596–1603.

Purkayastha, S., Kaur, B., Dilbaghi, N., Chaudhury, A. (2006): Characterization of *Macrophomina phaseolina*, the charcoal rot pathogen of cluster bean, using conventional techniques and PCR-based molecular markers. *Plant Pathol.,* **55**, 106–116.

Vajna, L., Rozsnyai, Z. (1995): *Macrophomina phaseolina* (Tassi) Goid. és a *Diaporthe eres* Nitschke, mint a fiatal kajszifák elhalásában szerepet játszó gombák Magyarországon. [*Macrophomina phaseolina* (Tassi) Goid. and *Diaporthe eres* Nitschke as causal agents of decline of young apricot trees in Hungary.] *Növényvédelem,* **31**, 81–83.

Zándoki, E., Sződi, S., Turóczi, G. (2005): Mycelial compatibility of *Sclerotinia sclerotiorum* strains of different area. *Acta Phytopathol. Entomol. Hung.,* **40**, 295–301.

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Review

REVIEW OF GENETIC DIVERSITY STUDIES IN ALMOND (*Prunus dulcis*)

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Almond [*Prunus dulcis* (Mill.) D.A. Webb.] is cultivated for its nuts and is one of the oldest domesticated plants. Due to the genetically controlled self-incompatibility system that operates in this species, almond is characterized by great genetic diversity, the estimation of which has benefited from a range of marker techniques, including morphological characteristics, isoenzyme detection and molecular markers. Among the DNA-based molecular markers, simple sequence repeats (SSR) have been used most widely, although analyses have ranged from restriction fragment length polymorphism to the most recent single nucleotide polymorphism detection methods. Molecular markers have also been used to trace specific agronomic traits, e.g. self-(in)compatibility or kernel bitterness. Genetic diversity studies in almond have not revealed a direct relationship between the level of diversity and the origin of the germplasm. This might be explained by the relatively recent occurrence of self-compatibility in almond, which has not yet caused a serious loss of genetic diversity. The markers reviewed will be useful in monitoring and maintaining genetic diversity in almond breeding programmes, while others may permit marker-assisted selection for favourable agronomic traits. The cultivation, breeding and conservation of wild-growing almonds may equally benefit from the genetic diversity studies (especially those applying molecular markers).

Key words: almond, crop evolution, genetic diversity, marker-assisted selection, molecular markers, microsatellite, self-(in)compatibility, SSR

Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb. syn. *P. amygdalus* Batsch] is a member of the *Rosaceae* family, *Prunoideae* subfamily. The *Rosaceae* family is one of the most important plant families in the temperate zone and includes a number of economically important species (e.g. apple, apricot, plum, sweet and sour cherry, almond, strawberry and rose). Almond is commercially grown worldwide for its nuts. The putative origin of almond is in the arid mountainous regions of Central Asia (Grasselly, 1976; Arús et al., 2009), and several wild

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species grow in areas ranging from Tian-shan through Afghanistan into Iran and Iraq (Grasselly, 1976; Kester and Gradziel, 1996). The *Prunus* species *P. fenzliana* (Fritsch) Lipsky, *P. bucharica* Korschinsky and *P. kuramica* Korschinsky are described as the wild species most closely related to cultivated almond in these regions (Grasselly, 1976; Kester et al., 1991; Browicz and Zohary, 1996). However, Ladizinsky (1999) identified only *P. fenzliana* as the wild ancestor of almond. Another putative ancestor species of cultivated almond is *P. webbii* (Spach) Vieh., which is thought to have originated on the Balkan Peninsula and seems to be closely related to almond. Three stages can be distinguished in the evolution and distribution of almonds: Asiatic, Mediterranean and Californian, according to the geographical areas where the species is grown (Grasselly, 1976; Kester et al., 1991; Kester and Gradziel, 1996; Martínez-Gómez et al., 2007).

Almond shows gametophytic self-incompatibility (de Nettancourt, 2001), which is controlled by the highly polymorphic, multiallelic *S*-locus. The *S*-locus encodes for an *S*-ribonuclease (*S*-RNase) protein in the pistils, which degrades RNA in self-pollen tubes and hence stops their growing (McClure et al., 1989). Consequently, almond is a typically outbreeding species, which has dramatic consequences on the genetic diversity of both wild growing and cultivated accessions, similarly to other tree fruit species (Halász et al., 2011; Hegedűs et al., 2011).

The term 'genetic marker' is associated with a gene resulting in a visible phenotype or characteristics, or a non-coding part of the genome (which does not contribute to the phenotype). The analysis of almond genetic variability can be performed by tracing morphological traits or applying various biochemical or molecular marker techniques ranging from isoenzyme detection to DNA analysis. The latter includes restriction fragment length polymorphism (RFLP) and several PCR-based techniques. Among these techniques, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple-sequence repeats (SSR), inter-simple sequence repeats (ISSR), single nucleotide polymorphisms (SNP) and functional markers (detecting the molecular changes behind a specific phenological trait) have been used for almond analysis. The aim of this study was to give an overview of the results of all possible marker strategies used for almond genetic diversity studies and to estimate their applicability in breeding. The data were also processed to show what these studies can tell us about the origin and evolution of cultivated almond.

Morphological traits

Surveying morphological traits was the first strategy used for the identification and characterization of almond cultivars, but these traits are not

really adequate for such analyses as they are affected by changing environmental conditions and may only be visible in adult material.

Browicz and Zohary (1996) studied the taxonomic relationships, morphological distinction (growth habit, leaf shape, leaf margins, petiole length, flower colour, length, fruit shape, etc.), geographical distribution and ecological specificities of 26 *Amygdalus* L. species (recognized as such by these authors). Five groups of closely related vicarious *Amygdalus* species were distinguished, including the *Communis* group with 9 species, the *Orientalis* group with 6 species, Sect. *Chamaeamygdalus* with 4 species, Sect. *Spartioides* with 2 species, and Subgenus *Dodecandara* with 5 species. Interspecific sterility barriers are absent or only weakly developed between these species, so numerous inter-specific hybrids have been detected.

Fathi et al. (2008) assessed the genetic diversity of *P. dulcis* using 14 morphological traits and SSR markers. The analysis revealed a wide range of phenotypic variation among the genotypes studied. The results of the study also showed that SSR markers could be successfully used to identify informative markers for improving traits in breeding programmes.

Zeinalabedini et al. (2010) examined four wild Iranian *Prunus* species, namely *P. eleagnifolia* Spach, *P. hausskenchtii* (C. Schneider) Bornm., *P. scoparia* Spach and *P. lycoides* Spach. They used morphological traits, protein and DNA markers in the genetic characterization and made a critical comparison between these techniques. Among the morphological traits, only leaf width showed significant differences in the four wild *Prunus* species.

Isoenzyme detection

Isoenzymes differ in their amino acid sequences but catalyse the same biochemical reaction. Due to the structural differences, polyacrylamide gel electrophoresis (PAGE) separates them according to their sizes or isoelectric points (isoelectric focusing). Isoenzymes may be encoded by genes at different loci or different alleles at the same locus (known as alloenzymes or allozymes). The detection of isoenzymes was the first molecular marker strategy to be used, because of their co-dominant expression and good reproducibility (Arulsekar et al., 1986; Hauagge et al., 1987a, b; Cerezo et al., 1989; Foolad et al., 1995; Vezvaei et al., 1995; Sathe et al., 2001). Isozyme studies have detected high levels of genetic variability in almond and allowed the individual identification of most genotypes studied. A comparative study of isoenzyme variability (including glucose phosphate isomerase, leucine aminopeptidase, aspartate aminotransferase, malate dehydrogenase, etc.) in *Prunus* showed that almond and Japanese plum, both having a strong self-incompatibility system, were characterized by higher variability at isoenzyme loci than species (e.g. apricot and peach) that have many self-compatible cultivars (Byrne, 1990). Nevertheless, the utilization of isoenzyme detection is limited by the small

number of loci that can be analysed with relatively simple enzyme staining methods, as well as the low levels of allelic variation at most loci. Interestingly, as a biochemical parameter the total protein content seemed to be accurately correlated with the taxonomical relationships between various wild relatives of almond (Zeinalabedini et al., 2008).

Linkage analysis was first performed in almond based on isoenzyme genes (Arús et al., 1994; Vezvaei et al., 1995), but the low number of isoenzymes that can be analysed with conventional enzyme staining methods in a population precluded the use of these markers for the construction of genetic maps. The first map for almond was constructed by Viruel et al. (1995) using a combination of isoenzyme and DNA-based markers (seven isoenzymes and 120 RFLPs) for the F_1 progeny of a cross between Ferragnes and Tuono (the F×T map).

Molecular markers

Molecular markers are nucleotide variations in a specific region of the DNA molecule, which show Mendelian inheritance. A marker may be a gene, but is sometimes a DNA region without any known function. The DNA-based molecular markers provide several advantages over other approaches: they are fast, accurate, highly discriminative, environmentally stable assays and allow for the early selection of specific characteristics as linked or functional markers. Early selection is of crucial importance in breeding programmes since tree fruits are characterized by a long juvenile phase, and many features (connected with flowers and fruit) are only expressed when the trees are 3–6 years old. Besides marker-assisted selection, molecular markers have been used in almond for genetic variability analyses, pedigree determinations or cultivar identification (Wünsch and Hormaza, 2002; Martínez-Gómez et al., 2003b; Sánchez-Pérez et al., 2004).

Various marker systems have been applied for genetic studies in wild-growing and cultivated almonds, including RFLP, RAPD, AFLP, SSR, ISSR and SNP analyses. The relative advantages and disadvantages of these techniques are summarized in Table 1 according to Agarwal et al. (2008) with modifications.

RFLP markers are co-dominant and detect a virtually unlimited number of markers, thus providing an efficient method for discovering relationships between cultivars or species. However, due to notable drawbacks (e.g. it is laborious and time-consuming and often involves the use of radioisotopes), RFLP analysis has only rarely been used for genetic variability analysis and cultivar identification in almond (Viruel, 1995).

PCR-based markers increased the opportunities for both diversity studies and mapping a wide range of traits. The most important advantage of such markers is that detection using isotopes was replaced by the widely available PCR amplification. The RAPD method is based on PCR and typically uses arbitrary primers that have random locations in the genome. Since single short oligonucleotides may amplify several parts of the genomic DNA, RAPD provides dominant markers characterized by variable degrees of repeatability.

This marker strategy has been widely used in almond, especially for genetic variability studies.

Table 1

Comparison of frequently used molecular marker techniques, modified after Agarwal et al. (2008)

Features	RFLP	RAPD	AFLP	SSR	ISSR	SNP
Frequency	high	high	high	moderate	moderate	moderate
Reproducibility	high	unreliable	high	high	high	high
Degree of polymorphism	moderate	moderate	moderate	moderate	moderate	moderate
PCR-based	no	yes	yes	yes	yes	yes
Specification of locus	yes	no	no	no	no	no
Development cost	low	low	moderate	high	low	high
DNA required (μg)	10	0.02	0.5–1.0	0.05	0.05	0.05
DNA quality	high	high	moderate	moderate	moderate	high
Ease to use	not easy	easy	easy	easy	easy	easy
Cost per analysis	high	low	moderate	low	low	low
Amenable to automation	low	moderate	moderate	high	moderate	high
Expression	co-dominant	dominant	dominant	co-dominant	dominant	co-dominant
Predominant application	physical mapping	gene tagging		analysis of genetic diversity		
No. of studies in almond[a]	1	6	2	8	2	1

[a]Number of studies found in international literature and used in the review

Californian almond cultivars were developed from highly selected subgroups of the central and southwest Asian populations. The genetic relatedness between 17 almond genotypes was estimated using 37 RAPD markers (Bartolozzi et al., 1998). Genetic diversity between the cultivars was limited despite the out-crossing nature of almond. A similarity index based on the proportion of shared fragments showed relatively high levels of 0.75 or greater. As expected, the level of similarity between almond and peach was much lower.

Martins et al. (2003) analysed the genetic diversity of 30 Portuguese and 10 foreign *P. dulcis* cultivars and their relationships by RAPD and inter-simple sequence repeat (ISSR) markers. They also studied five unidentified almond plants in the region of Foz Côa (north Portugal) and six wild *P. webbii* accessions from Italy and Spain. More than 70 primers (RAPD and ISSR) were used in this experiment for six RAPD and five ISSR analyses, providing good reproducibility and high polymorphism. Out of the 124 PCR fragments, 120 were polymorphic. A dendrogram based on the Dice similarity coefficient and the clustering algorithm of the unweighted pair group method with arithmetic averages (UPGMA) distinguished four main groups among the tested plants: *P. dulcis* cultivars, one Foz Côa plant, *P. webbii* and *P. persica* (L.) Batsch (used as an outgroup). The Foz Côa plant was hypothesized to be a hybrid between *P. dulcis* and *P. webbii*. The genetic diversity of the *P. dulcis* cultivars was high (the estimated similarity coefficients, based on Dice values, ranged between 0.97 and 0.65) and it was possible to discriminate all the almond cultivars analysed.

The genetic variability of the Portuguese almond cultivars was identical to that of the *P. dulcis* cluster, making Portuguese cultivars a very heterogeneous group.

Later, Martins et al. (2004) used the same set of RAPD and ISSR markers to characterize the genetic stability of micropropagated almond plantlets. They analysed an almond clone, designated clone VII, derived from a seedling of the cultivar Boa Casta. To evaluate the somaclonal variation in this clone, they compared the RAPD and ISSR patterns of plantlets obtained after 4 and 6 years of *in vitro* multiplication. A total of 7,172 bands were scored showing monomorphic patterns across all the plantlets and hence indicating their genetic identity. The results confirmed that the culture conditions used for axillary branching are appropriate for the clonal propagation of almond clones, since they did not interfere with the integrity of the regenerated plantlets.

A total of 22 Tunisian and one Moroccan almond cultivars were analysed using 12 RAPD markers and compared with Mediterranean and USA cultivars (Gouta et al., 2008). All the primers revealed polymorphism and the number of polymorphic bands varied from 6 to 13. The great genetic diversity within the almond genotypes (similarity values ranging from 0.45 to 0.94) was explained by the self-incompatibility of the majority of cultivars and the varying climates found within Tunisia. The UPGMA tree divided the cultivars into six groups, the most distant of which formed an outgroup containing the cultivars Laurane and Genco. The RAPD markers also identified the mislabelling of a genotype.

MirAli and Nabulsi (2003) analysed 19 almond cultivars (eight native Syrian and 11 foreign) grown at two germplasm collections in Syria using RAPD markers. They used forty primers, all but one of which produced polymorphism. The similarity matrix showed that the tested genotypes had limited genetic diversity (with an average similarity index of 0.78). The cluster analysis divided the genotypes into two groups, with two cultivars (one Iranian and the French cultivar Ferralise) distantly related to both groups. UPGMA clustering based on RAPD markers agreed with the geographical origins of the Syrian cultivars analysed.

Iranian almond cultivars and their relationship to foreign cultivars and to three related species were studied by Shiran et al. (2007) using RAPD markers. They analysed 12 Iranian genotypes, 24 foreign accessions and three wild almond species [*Prunus communis* (L.) Arcang., *P. orientalis* (Mill.) Koehne and *P. scoparia*]. Out of the 80 RAPD primers, 42 were highly polymorphic and reproducible. A total of 664 polymorphic RAPD bands were detected. Both the RAPD and SSR techniques discriminated the genotypes efficiently, although only the RAPD primers were able to distinguish the cultivars Monagha and Sefied. The results showed high genetic variability within the tested cultivars. The UPGMA dendrogram topologies for the two markers also revealed great similarity. Both dendrograms reflected the relationships between the cultivars and species according to their geographic origins and/or pedigree information. The almond cultivars clustered with the *P. communis* accession, showing their

close relationship. *P. orientalis* and *P. scoparia* clustered separately from *P. dulcis*.

Amplified fragment length polymorphism (AFLP) analysis is a rapid and efficient method, developed in the early 1990s by Keygene and described by Vos et al. (1995). This technique is an adequate PCR-based tool used in DNA fingerprinting and molecular characterization. AFLP is a highly sensitive method for detecting polymorphisms in DNA, using restriction enzymes to digest genomic DNA, followed by the ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments is then selected to be amplified. This selection is achieved using primers complementary to the adaptor sequence, the restriction site sequence and a few arbitrarily chosen nucleotides to decrease the number of amplified fragments. The amplified fragments are then visualized on denaturing polyacrylamide gels, either through autoradiography or fluorescence labelling.

Martins et al. (2001) compared 40 Portuguese almond accessions with several markers. AFLP revealed high levels of polymorphism between the PCR markers, and in terms of reproducibility AFLP again proved to be a reliable technique. Hence, Sorkheh et al. (2007) estimated the genetic similarities, marker indices and polymorphic information contents (PICs) of eight Iranian and 28 European and US almond cultivars using AFLP markers. They also evaluated several agronomic traits (flowering time, maturity time, self-incompatibility, kernel and fruit properties). Most (96%) of the fragments were polymorphic, with genetic similarities ranging from 0.5 to 0.96. The correlation between genetic similarity clustering based on AFLP and clustering for agronomic traits was low. Both the AFLP results and morphology suggest that the Iranian almond germplasm shares a common genetic background, differing in genotype and morphology from the French, US, and Spanish cultivars. AFLP markers revealed great genetic diversity among the almond cultivars, which might be exploited in breeding.

SSR or microsatellite markers are also based on PCR, and have become the most widely used markers for the genetic fingerprinting of a wide range of plants. Because of their high polymorphism, abundance and co-dominant inheritance, they are well suited for the assessment of genetic variability within crop species and genetic relationships between closely related species (Gupta et al., 1996; Powell et al., 1996). In the case of *Prunus*, SSR markers covering almost the whole genome have been obtained in different species including peach, apricot, Japanese plum and cherry (Cipriani et al., 1999; Downey and Iezzoni, 2000; Sosinski et al., 2000; Testolin et al., 2000; Cantini et al., 2001; Aranzana et al., 2002; Dirlewanger et al., 2002; Georgi et al., 2002; Wang et al., 2002; Yamamoto et al., 2002; Aranzana et al., 2003; Clarke and Tobutt, 2003; Decroocq et al., 2003; Schueler et al., 2003; Hagen et al., 2004; Messina et al., 2004; Mnejja et al., 2004). The first set of almond SSRs was published by Testolin et al. (2004). These primers were used for the molecular

characterization and identification of almond cultivars (Martínez-Gómez et al., 2003a; Testolin et al., 2004). Martínez-Gómez et al. (2003a) analysed 30 *P. dulcis* and 35 *P. persica* cultivars using SSR markers. Heterozygosity was much higher in almond cultivars (0.38–0.88) in comparison with peach cultivars (0.05–0.33), coinciding with the different mating strategies of these species, as almonds are predominantly self-incompatible while peach is an exclusively self-compatible species. These results gave an unequivocal verification of the data supplied by former isoenzyme analyses (Arulsekar et al., 1986). Two major clusters were also observed in almond, with one containing Californian cultivars and the other containing mainly European cultivars. These results established the value of SSR markers in distinguishing different almond genetic lineages.

Later, for the SSR analysis of Californian almond cultivars, Dangl et al. (2009) proposed a set of 12 primers, capable of discriminating closely related cultivars. A comprehensive study comparing 93 almond genotypes (including 63 Spanish cultivars) was also carried out (Fernandez i Marti et al., 2009). The observed heterozygosity (0.72) was higher than in previous studies on almond germplasm.

For Tunisian almonds, a study by Gouta et al. (2010) using ten almond SSR primers led to very similar conclusions to those drawn in an earlier RAPD analysis (Gouta et al., 2008). The mean observed heterozygosity was 0.68. In addition to the separation of northern and southern cultivars already achieved by RAPD, a clear distinction was demonstrated between central and southern cultivars and all the others tested.

Fifty-one Iranian and Azerbaijani cultivars and landraces were analysed using 25 polymorphic SSR primers and their genetic relationships were depicted on a UPGMA dendrogram. The almond genotypes were classified into five main groups. Iranian cultivars were also studied by Shiran et al. (2007). Out of the 26 SSR primers, 18 were polymorphic. The number of presumed alleles revealed by the SSR analysis ranged from 3 to 10 alleles per locus. The heterozygosity level of the cultivars ranged from 0.29 to 0.71, with no difference in the range of heterozygosity for Iranian and other Mediterranean and US cultivars.

Xie et al. (2006) examined the genetic diversity of sixteen (8 EST-SSR and 8 genomic SSR) loci in 23 Chinese and 15 international almond cultivars. The genomic SSR primers, previously reported mainly in peach, proved to be useful for genetic analysis in almond. The EST-SSRs were developed in almond. The DNA sequences of 117 alleles of six of the 16 SSR loci were determined in order to reveal sequence variation among the 38 almond accessions. In 98 of the sequenced alleles, no insertions or deletions were observed in the flanking regions. Some of the analysed alleles had a high number of uninterrupted repeat motifs, showing that the SSR mutational patterns differ for individual alleles at a given SSR locus in almond. Allelic homoplasy (sequence variation that does not cause allelic size change) was observed in the SSR loci due to base substitutions, interruptions or compound repeat motifs. Therefore, the actual level of polymorphism in almond may be underestimated if based only on SSR allele

size variation. Substitutions were also found at two SSR loci in the repeat regions, pointing to a putative accumulation of point mutations in the SSRs, which will consequently inhibit further SSR expansion by introducing repeat interruptions and thus stabilize SSR loci. Such analyses may help to elucidate the evolutionary processes behind SSR data.

Nuclear SSR markers were the most abundant markers, with higher polymorphism compared to morphological, protein and chloroplast SSR markers (Zeinalabedini et al., 2010). A combined nuclear and chloroplast SSR analysis showed *P. fenzliana* to have the closest genetic relationship with cultivated almond, supporting the hypothesis of Ladizinsky (1999), which was based on morphological traits.

High resolution melting analysis (HRM) is a recent advance for the detection of mutations, SNPs, polymorphisms and epigenetic differences in distinct DNA samples. This technique has some advantages over other genotyping assays, being cost-effective, fast, simple and powerful and thus suitable for high-throughput, accurate analysis. However, real-time PCR and new generation fluorescent dyes are required for the analysis. Wu et al. (2008) analysed almond SNPs derived from ESTs assessed by the HRM method and determined the temperature that induces the separation of strands of short PCR amplicons. The analysis detected variations as small as one base difference between the samples.

Wu et al. (2008) applied HRM analysis for the discovery of almond SNPs and for genotyping based on the predicted SNP information derived from almond and peach EST databases. They analysed 25 almond cultivars and screened for putative SNPs through HRM analysis. The HRM profiles of 17 amplicons were established and all four classes of SNPs, INDELs and microsatellites were discriminated. Various genotypes of INDEL and microsatellite variations were also characterized using the HRM technique. The sequencing of the amplified PCR products revealed 100 SNPs in the HRM amplicons and their flanking regions. The average frequency of SNPs was 1:114 bp in the genic regions, while the ratio of transition to transversion was 1.16:1. The rare allele frequencies of the SNPs varied from 0.02 to 0.5, and the average polymorphic information content of the SNPs was 0.31. HRM has been demonstrated to be a fast, low-cost, efficient approach for SNP discovery and genotyping, in particular for species without detailed genomic information, such as almond.

Functional markers and marker-assisted selection

The three main objectives of almond improvement are to increase yield (self-compatibility, late flowering, flower density, and productivity), improve quality (maturity date, kernel taste), and decrease production costs (resistance to biotic and abiotic stress factors) (Socias i Company, 1998). Below, two of the above-mentioned traits, self-incompatibility and kernel bitterness, are discussed in detail as examples of functional markers and SSR marker-assisted selection, respectively.

The history of the analysis of self-incompatibility in almond clearly follows the evolutionary process of developing marker strategies in tree fruit species. First, through phenotypical investigation (open field pollinations and evaluation of the resulting fruit set), four self-incompatibility alleles were determined (Tufts and Philp, 1922). The greatest disadvantage of this procedure is that *S*-genotyping can only be achieved by a series of crosses, which takes many years due to the long juvenile phase of fruit trees. When the stylar component of the incompatibility system was found to be a ribonuclease enzyme, isoelectric focusing and subsequent specific staining for RNase activity made direct *S*-genotype determinations possible from the allozyme patterns of cultivars. Many *S*-RNase alleles were identified in almond using this approach (Boskovic et al., 1997; 2003). However, this analysis still required flowering material (several years old). PCR-based strategies have also been developed for the identification of *S*-RNase alleles using genomic DNA (Tamura et al., 2000; Ushijima et al., 2003). Many almond *S*-RNase alleles have been cloned and sequenced (Ortega et al., 2006; Kodad et al., 2008; Halász et al., 2010a) using consensus primers (Sutherland et al., 2004). In this case, allele detection is based on the allele-specific size variations of both *S*-RNase introns. However, primers for allele-specific amplification were also designed to discriminate between alleles with similar intron lengths (Halász et al., 2008). The number of *S*-alleles also indicated the great genetic diversity of almonds ranging all the way from the USA to Eastern Europe.

All these primers could be used as functional markers, allowing the early selection of self-incompatibility genotypes in breeding programmes. However, a more important trait of interest in breeding programmes is self-compatibility. Self-compatible almonds carry the S_f-RNase allele, which does not code for ribonuclease activity. Hence, a co-dominant stylar ribonuclease assay was developed, where genotypes having only one *S*-RNase isoenzyme were assumed to be heterozygotes (S_fS_i; S_f: allele for self-compatibility, S_i: allele for self-incompatibility), while those having no active *S*-RNases were homozygous, self-compatible (S_fS_f) accessions (Boskovic et al., 1999). Later, alleles with coding and 5′ regulatory sequences identical to S_f were also isolated from self-incompatible cultivars, showing that the different expression of S_f is independent of the complete genetic identity found in the whole chromosome region bordering the *S*-locus (Fernández i Marti et al., 2010). It is also evident that a reliable DNA-based molecular marker must be associated with sequences outside the *S*-locus, and hence some previous RFLP markers (Table 2) should be tested on incompatible cultivars carrying the S_f-allele.

To avoid kernel bitterness is a major challenge in almond breeding. The trait is sporophytically controlled, so all the nuts on a tree will have either bitter or sweet kernels (Dicenta et al., 2000). Bitterness is induced by the content of amygdalin, which is degraded to glucose, benzaldehyde (which confers a bitter flavour) and hydrogen cyanide, a toxic compound. The trait is monofactorial, determined by the *Sk* (*Sweet kernel*) locus, with the sweet allele being dominant (*Sk*) to the bitter one (*sk*). The *Sk* gene was mapped to linkage group 5 (G5), but

none of the known candidate genes were found on G5. Therefore, Sánchez-Pérez et al. (2010) saturated G5 with SSR primers, four of which were closely linked to the *Sk* locus and proved suitable for early, efficient selection against bitter kernel in a breeding programme after estimating the haplotypes of the parents and the identification of appropriate marker alleles.

Synteny among *Prunus* genomes will greatly facilitate the successful transfer of sets of markers and coding sequences between species (Martínez-Gómez et al., 2007). From this point of view the peach genome that has been made available recently will be particularly useful (http://www.rosaceae.org/node/355).

Table 2
Markers associated with main agronomic traits in almond, modified after Martínez-Gómez et al. (2007)

Trait	Symbol	Linkage group	Marker	Reference
Flower colour	*B*	G1	RFLP	Jáuregui (1998)
Shell hardness	*D*	G2	RFLP	Arús et al. (1998)
Nematode resistance	*Mi*	G2	RFLP	Jáuregui (1998)
Nematode resistance	*Mi*	G2	RFLP	Bliss et al. (2002)
Fusicoccum resistance	*Fc*	?	SCAR/CAPS	Martins et al. (2005)
Anther colour	*Ag*	G3	RFLP	Joobeur (1998)
Blooming time	*Lb*	G4	RAPD	Ballester et al. (2001)
Kernel taste	*Sk*	G5	RFLP	Bliss et al. (2002)
Kernel taste	*Sk*	G5	RFLP	Joobeur (1998)
Kernel taste	*Sk*	G5	SSR	Sánchez-Pérez et al. (2010)
Self-incompatibility	*S*	G6	RAPD	Ballester et al. (2001)
Self-incompatibility	*S*	G6	Candidate gene	Sutherland et al. (2004)
Self-incompatibility	*S*	G6	Candidate gene	Halász et al. (2008)
Self-compatibility	*S*	G6	Isoenzyme	Boskovic et al. (1999)
Self-compatibility[a]	*S*	G6	RFLP	Ballester et al. (1998)
Self-compatibility[a]	*S*	G6	RFLP	Arús et al. (1998)
Self-compatibility[a]	*S*	G6	RFLP	Bliss et al. (2002)

[a]Determination of self-compatibility may only be valid if RFLP marker is associated with sequences outside the *S*-locus

Concluding remarks

The last decade showed that the molecular marker technique most intensively used and most appropriate for genetic diversity studies in almond was SSR analysis. SSR revealed higher genetic variability in almond than RAPD, demonstrating the efficiency of SSR analysis. Unfortunately, the data published by several research teams could not be directly translated into crop evolutionary statements because of the many discrepancies between the analyses, including the different primers used (Fernandez i Marti et al., 2009), the different number of genotypes tested and the different techniques for allele detection, such as automated sequencer or electrophoresis (Sánchez-Pérez et al., 2006). In addition, most papers compared local genotypes with internationally

popular cultivars, but neglected to report SSR diversity parameters separately for the different germplasms compared. In many cases, only data determined from the analysis of all the tested genotypes, with mixed origins, were shown.

However, if all the available data were considered, no correlations would be found between genetic diversity levels and the place of origin of the germplasm. In SSR analyses, the average observed heterozygosity was 0.72 in 63 Spanish cultivars (Fernandez i Marti et al., 2009), 0.69 in 23 Chinese cultivars (Xie et al., 2006) and 0.59 in 30 US and Mediterranean cultivars (Martínez-Gómez et al., 2003a). RAPD gave no characteristic differences, either. This tendency has nothing to do with the known dissemination routes of almonds from Central Asia to the Mediterranean, which could be explained by the discrepancies described above. However, genetic relationships calculated from the proportion of shared alleles gave a good reflection of the closer genetic relationships between Spanish, French, Italian and Iranian cultivars in comparison with Ukrainian, Slovakian and Czech cultivars (Zeinalabedini et al., 2008). Distinct clustering was also apparent for Portuguese and Tunisian cultivars.

Comparing this trend with that seen in another *Prunus* tree fruit, apricot, a different pattern emerges. In this species a continuous decrease in heterozygosity is evident from the Central Asian gene centre to the Western European growing countries. This phenomenon was found to be linked to changes in the mating strategy (Halász et al., 2007; 2010b), with self-compatibility arising putatively in the Eastern part of Turkey and then spreading rapidly into the Western regions, where it resulted in a heavy loss of genetic diversity (Pedryc et al., 2009). Self-compatibility was also described in some Italian and Spanish almond cultivars (Godini, 1979; Socias i Company, 2004), but without any consequences for genetic diversity. This might be explained by the different lengths of time for which the two species have been capable of inbreeding. In apricot, it dates back for several thousands of years, while self-compatibility in almond is a relatively new trait (spanning only a few decades), which has not yet had time to induce genetic erosion. In other words, the almond germplasm is currently in the stage where apricot germplasm was thousands of years ago. The history of apricot could be instructive for almond breeding, demonstrating the need to maintain the genetic diversity of the almond germplasm. The correspondence between genetic diversity and the mating strategy highlights how powerful an effect the self-(in)compatibility system has in shaping the genetic basis of a tree fruit.

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References

Agarwal, M., Shrivastava, N., Padh, H. (2008): Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Rep.,* **27**, 617–631.

Aranzana, M., Pineda, A., Cosson, P., Dirlewanger, E., Ascasibar, J., Cipriani, G., Ryder, C., Testolin, R., Abbott, A., King, G. J., Iezzoni, A. F., Arús, P. (2003): A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. *Theor. Appl. Genet.,* **106**, 819–825.

Aranzana, M. J., Garcia-Mas, J., Carbó, J., Arús, P. (2002): Development and variability analysis of microsatellite markers in peach. *Plant Breeding,* **121**, 87–92.

Arulsekar, S., Parfitt, D. E., Kester, D. E. (1986): Comparison of isozyme variability in peach and almond cultivars. *J. Hered.,* **77**, 272–274.

Arús, P., Ballester, J., Jáuregui, B., Joobeur, T., Truco, M., Carmen de Vicente, M. (1998): The European *Prunus* mapping project: update on marker development in almond. *Acta Hort.,* **484**, 331–338.

Arús, P., Gradziel, T., Oliveira, M. M., Tao, R. (2009): Genomics of almond. In: Folta, K. M., Gardiner, S. E. (eds.), *Genetics and Genomics of Rosaceae*, Vol. 6. Springer, New York, pp. 187–219.

Arús, P., Olarte, C., Romero, M., Vargas, F. (1994): Linkage analysis of ten isozyme genes in F_1 segregating almond progenies. *J. Amer. Soc. Hort. Sci.,* **119**, 339–344.

Ballester, J., Bošković, R., Batlle, I., Arús, P., Vargas, F., de Vicente, M. C. (1998): Location of the self-incompatibility gene on the almond linkage map. *Plant Breeding,* **117**, 69–72.

Ballester, J., Socias i Company, R., Arus, P., De Vicente, M. C. (2001): Genetic mapping of a major gene delaying blooming time in almond. *Plant Breeding,* **120**, 268–270.

Bartolozzi, F., Warburton, M. L., Arulsekar, S., Gradziel, T. M. (1998): Genetic characterization and relatedness among California almond cultivars and breeding lines detected by randomly amplified polymorphic DNA (RAPD) analysis. *J. Amer. Soc. Hort. Sci.,* **123**, 381–387.

Bliss, F. A., Arulsekar, S., Foolad, M. R., Becerra, V., Gillen, A. M., Warburton, M. L., Dandekar, A. M., Kocsisne, G. M., Mydin, K. K. (2002): An expanded genetic linkage map of *Prunus* based on an interspecific cross between almond and peach. *Genome,* **45**, 520–529.

Boskovic, R., Tobutt, K. R., Batlle, I., Duval, H. (1997): Correlation of ribonuclease zymograms and incompatibility genotypes in almond. *Euphytica,* **97**, 167–176.

Boskovic, R., Tobutt, K. R., Batlle, I., Duval, H., Martinez-Gomez, P., Gradziel, T. M. (2003): Stylar ribonucleases in almond: correlation with and prediction of incompatibility genotypes. *Plant Breeding,* **122**, 70–76.

Boskovic, R., Tobutt, K. R., Duval, H., Batlle, I., Dicenta, F., Vargas, F. J. (1999): A stylar ribonuclease assay to detect self-compatible seedlings in almond progenies. *Theor. Appl. Genet.,* **99**, 800–810.

Browicz, K., Zohary, D. (1996): The genus *Amygdalus* L. (*Rosaceae*): Species relationships, distribution and evolution under domestication. *Genet. Resour. Crop Ev.,* **43**, 229–247.

Byrne, D. H. (1990): Isozyme variability in four diploid stone fruits compared with other woody perennial plants. *J. Hered.,* **81**, 68–71.

Cantini, C., Iezzoni, A. F., Lamboy, W. F., Boritzki, M., Struss, D. (2001): DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats. *J. Amer. Soc. Hort. Sci.,* **126**, 205–209.

Cerezo, M., Socias i Company, R., Arus, P. (1989): Identification of almond cultivars by pollen isoenzymes. *J. Amer. Soc. Hort. Sci.,* **114**, 164–169.

Cipriani, G., Lot, G., Huang, W. G., Marrazzo, M. T., Peterlunger, E., Testolin, R. (1999): AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. *Theor. Appl. Genet.,* **99**, 65–72.

Clarke, J. B., Tobutt, K. R. (2003): Development and characterization of polymorphic microsatellites from *Prunus avium* 'Napoleon'. *Mol. Ecol. Notes,* **3**, 578–580.

Dangl, G., Yang, J., Golino, D., Gradziel, T. (2009): A practical method for almond cultivar identification and parental analysis using simple sequence repeat markers. *Euphytica,* **168**, 41–48.

de Nettancourt, D. (2001): *Incompatibility and Incongruity in Wild and Cultivated Plants* (2nd ed.). Springer-Verlag, New York.

Decroocq, V., Favé, M. G., Hagen, L., Bordenave, L., Decroocq, S. (2003): Development and transferability of apricot and grape EST microsatellite markers across taxa. *Theor. Appl. Genet.,* **106**, 912–922.

Dicenta, F., Martinez-Gomez, P., Ortega, E., Duval, H. (2000): Cultivar pollinizer does not affect almond flavor. *HortScience,* **35**, 1153–1154.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M., Poizat, C., Zanetto, A., Arús, P., Laigret, F. (2002): Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theor. Appl. Genet.,* **105**, 127–138.

Downey, S. L., Iezzoni, A. F. (2000): Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. *J. Amer. Soc. Hort. Sci.,* **125**, 76–80.

Fathi, A., Ghareyazi, B., Haghnazari, A., Ghaffari, M. R., Pirseyedi, S. M., Kadkhodaei, S., Naghavi, M. R., Mardi, M. (2008): Assessment of the genetic diversity of almond (*Prunus dulcis*) using microsatellite markers and morphological traits. *Iran J. Biotechnol.,* **6**, 98–106.

Fernandez i Marti, A., Alonso, J. M., Espiau, M. T., Rubio-Cabetas, M. J., Socias i Company, R. (2009): Genetic diversity in Spanish and foreign almond germplasm assessed by molecular characterization with simple sequence repeats. *J. Amer. Soc. Hort. Sci.,* **134**, 535–542.

Fernández i Martí, Ŕ., Hanada, T., Alonso, J. M., Yamane, H., Tao, R., Socias i Company, R. (2010): The almond S_f haplotype shows a double expression despite its comprehensive genetic identity. *Sci. Hortic.-Amsterdam,* **125**, 685–691.

Foolad, M. R., Arulsekar, S., Becerra, V., Bliss, F. A. (1995): A genetic map of *Prunus* based on an interspecific cross between peach and almond. *Theor. Appl. Genet.,* **91**, 262–269.

Georgi, L., Wang, Y., Yvergniaux, D., Ormsbee, T., Iñigo, M., Reighard, G., Abbott, A. (2002): Construction of a BAC library and its application to the identification of simple sequence repeats in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.,* **105**, 1151–1158.

Godini, A. (1979): Ipotesi sulla comparsa dell'autocompatibilità nel mandorlo. (Hypotheses on the appearance of self-compatibility in almond.) *Riv. Sci. Tecn. Agrar.,* **19**, 3–10.

Gouta, H., Ksia, E., Zoghlami, N., Zarrouk, M., Mliki, A. (2008): Genetic diversity and phylogenetic relationships among Tunisian almond cultivars revealed by RAPD markers. *J. Hortic. Sci. Biotech.,* **83**, 707–712.

Gouta, H., Ksia, E., Buhner, T., Moreno, M. Á., Zarrouk, M., Mliki, A., Gogorcena, Y. (2010): Assessment of genetic diversity and relatedness among Tunisian almond germplasm using SSR markers. *Hereditas,* **147**, 283–292.

Grasselly, C. (1976): Origine et évolution de l'amandier cultivé. (Origin and evolution of cultivated almond.) *Opt. Méditerr.,* **32**, 45–50.

Gupta, P. K., Balyan, I. S., Sharma, P. C., Ramesh, B. (1996): Microsatellites in plants: A new class of molecular markers. *Curr. Sci. India,* **70**, 45–54.

Hagen, L. S., Chaib, J., Fady, B., Decroocq, V., Bouchet, J. P., Lambert, P., Audergon, J. M. (2004): Genomic and cDNA microsatellites from apricot (*Prunus armeniaca* L.). *Mol. Ecol. Notes,* **4**, 742–745.

Halász, J., Fodor, Á., Hegedus, A., Pedryc, A. (2008): Identification of a new self-incompatibility allele (S_{31}) in a Hungarian almond cultivar and its reliable detection. *Sci. Hortic.-Amsterdam,* **116**, 448–451.

Halász, J., Fodor, Á., Pedryc, A., Hegedus, A. (2010a): *S*-genotyping of Eastern European almond cultivars: identification and characterization of new (S_{36}–S_{39}) self-incompatibility ribonuclease alleles. *Plant Breeding,* **129**, 227–232.

Halász, J., Hegedus, A., György, Z., Pállinger, É., Tóth, M. (2011): *S*-genotyping of old apple cultivars from the Carpathian Basin: methodological, breeding and evolutionary aspects. *Tree Genet. Genomes,* in press, DOI: 10.1007/s11295-11011-10401-11297.

Halász, J., Pedryc, A., Ercisli, S., Yilmaz, K. U., Hegedus, A. (2010b): *S*-genotyping supports the genetic relationships between Turkish and Hungarian apricot germplasm. *J. Amer. Soc. Hort. Sci.,* **135**, 410–417.

Halász, J., Pedryc, A., Hegedus, A. (2007): Origin and dissemination of the pollen-part mutated S_C-haplotype which confers self-compatibility in apricot (*Prunus armeniaca*). *New Phytologist,* **176**, 792–803.

Hauagge, R., Kester, D. E., Arulsekar, S., Parfitt, D. E., Liu, L. (1987a): Isozyme variation among California almond cultivars. II. Cultivar characterization and origins. *J. Amer. Soc. Hort. Sci.,* **112**, 693–698.

Hauagge, R., Kester, D. E., Asay, R. A. (1987b): Isozyme variation among California almond cultivars: I. Inheritance. *J. Amer. Soc. Hort. Sci.,* **112**, 687–693.

Hegedűs, A., Lénárt, J., Halász, J. (2011): Review of sexual incompatibility in tree fruit species: molecular interactions and evolutionary dynamics. *Biol. Plantarum,* in press.

Jáuregui, B. (1998): *Localización de marcadores moleculares ligados a caracteres agronómicos en un cruzamiento interespecífico almendro melocotonero.* (Localisation of molecular markers linked to agronomic traits in an interspecific almond-peach cross.) Universitat de Barcelona, Barcelona, Spain.

Joobeur, T. (1998): *Construcción de un mapa de marcadores moleculares y análisis genético de caracteres agronómicos en Prunus.* (Construction of a molecular marker map and the genetic analysis of agronomic traits in *Prunus*.) Unpublished Doctoral Thesis, Universitat de Lleida, Lleida, Spain.

Kester, D. E., Gradziel, T. M. (1996): Almonds (*Prunus*). In: Moore, J. N., Janick, J. (eds.), *Fruit Breeding.* Wiley & Sons, New York, pp. 1–97.

Kester, D. E., Gradziel, T. M., Grasselly, C. (1991): Almonds (*Prunus*). *Acta Hort.,* **290**, 701–760.

Kodad, O., Sánchez, A., Saibo, N., Oliveira, M., Socias i Company, R. (2008): Identification and characterization of new *S*-alleles associated with self-incompatibility in almond. *Plant Breeding,* **127**, 632–638.

Ladizinsky, G. (1999): On the origin of almond. *Genet. Res. Crop Ev.,* **46**, 143–147.

Martínez-Gómez, P., Arulsekar, S., Potter, D., Gradziel, T. M. (2003a): An extended interspecific gene pool available to peach and almond breeding as characterized using simple sequence repeat (SSR) markers. *Euphytica,* **131**, 313–322.

Martínez-Gómez, P., Sánchez-Pérez, R., Dicenta, F., Howad, W., Arús, P., Gradziel, T. (2007): Almond. In: Kole, C. (ed.), *Fruits and Nuts*, Vol. 4. Springer, Berlin Heidelberg, pp. 229–242.

Martínez-Gómez, P., Sozzi, G. O., Sánchez-Pérez, R., Rubio, M., Gradziel, T. M. (2003b): New approaches to *Prunus* tree crop breeding. *J. Food Agric. Environ.,* **1**, 52–63.

Martins, M., Farinha, A., Ferreira, E., Cordeiro, V., Monteiro, A., Tenreiro, R., Oliveira, M. (2001): Molecular analysis of the genetic variability of Portugese almond collections. *Acta Hort.,* **546**, 449–452.

Martins, M., Sarmento, D., Batlle, I., Vargas, F. J., Oliveira, M. M. (2005): Development of SCAR/CAPS markers linked to tolerance/sensitivity to *Fusicoccum* in almond. *Opt. Méditerr.,* **63**, 187–191.

Martins, M., Sarmento, D., Oliveira, M. M. (2004): Genetic stability of micropropagated almond plantlets, as assessed by RAPD and ISSR markers. *Plant Cell Rep.,* **23**, 492–496.

Martins, M., Tenreiro, R., Oliveira, M. M. (2003): Genetic relatedness of Portuguese almond cultivars assessed by RAPD and ISSR markers. *Plant Cell Rep.,* **22**, 71–78.

McClure, B. A., Haring, V., Ebert, P. R., Anderson, M. A., Simpson, R. J., Sakiyama, F., Clarke, A. E. (1989): Style self-incompatibility gene products of *Nicotiana alata* are ribonucleases. *Nature,* **342**, 955–957.

Messina, R., Lain, O., Marrazzo, M. T., Cipriani, G., Testolin, R. (2004): New set of microsatellite loci isolated in apricot. *Mol. Ecol. Notes,* **4**, 432–434.

MirAli, N., Nabulsi, I. (2003): Genetic diversity of almonds (*Prunus dulcis*) using RAPD technique. *Sci. Hortic.-Amsterdam,* **98**, 461–471.

Mnejja, M., Garcia-Mas, J., Howad, W., Badenes, M. L., Arús, P. (2004): Simple-sequence repeat (SSR) markers of Japanese plum (*Prunus salicina* Lindl.) are highly polymorphic and transferable to peach and almond. *Mol. Ecol. Notes,* **4**, 163–166.

Ortega, E., Boskovic, R. I., Sargent, D. J., Tobutt, K. R. (2006): Analysis of S-RNase alleles of almond (*Prunus dulcis*): characterization of new sequences, resolution of synonyms and evidence of intragenic recombination. *Mol. Genet. Genom.,* **276**, 413–426.

Pedryc, A., Ruthner, S., Hermán, R., Krska, B., Hegedus, A., Halász, J. (2009): Genetic diversity of apricot revealed by a set of SSR markers from linkage group G1. *Sci. Hortic.-Amsterdam,* **121**, 19–26.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., Rafalski, A. (1996): The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breeding,* **2**, 225–238.

Sánchez-Pérez, R., Ballester, J., Dicenta, F., Arús, P., Martínez-Gómez, P. (2006): Comparison of SSR polymorphisms using automated capillary sequencers, and polyacrylamide and agarose gel electrophoresis: Implications for the assessment of genetic diversity and relatedness in almond. *Sci. Hortic.-Amsterdam,* **108**, 310–316.

Sánchez-Pérez, R., Dicenta, F., Gradziel, T. M., Arús, P., Martínez-Gómez, P. (2004): Application of molecular markers in almond breeding programmes. *Nucis-Newsl.,* **12**, 9–12.

Sánchez-Pérez, R., Howad, W., Garcia-Mas, J., Arús, P., Martínez-Gómez, P., Dicenta, F. (2010): Molecular markers for kernel bitterness in almond. *Tree Genet. Genomes,* **6**, 237–245.

Sathe, S. K., Teuber, S. S., Gradziel, T. M., Roux, K. H. (2001): Electrophoretic and immunological analyses of almond (*Prunus dulcis* L.) genotypes and hybrids. *J. Agr. Food Chem.,* **49**, 2043–2052.

Schueler, S., Tusch, A., Schuster, M., Ziegenhagen, B. (2003): Characterization of microsatellites in wild and sweet cherry (*Prunus avium* L.) – markers for individual identification and reproductive processes. *Genome,* **46**, 95–102.

Shiran, B., Amirbakhtiar, N., Kiani, S., Mohammadi, S., Sayed-Tabatabaei, B. E., Moradi, H. (2007): Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. *Sci. Hortic.-Amsterdam,* **111**, 280–292.

Socias i Company, R. (1998): Fruit tree genetics at a turning point: the almond example. *Theor. Appl. Genet.,* **96**, 588–601.

Socias i Company, R. (2004): The contribution of *Prunus webbii* to almond evolution. *Plant Genet. Res. Newsl.,* **140**, 9–13.

Sorkheh, K., Shiran, B., Gradziel, T., Epperson, B., Martínez-Gómez, P., Asadi, E. (2007): Amplified fragment length polymorphism as a tool for molecular characterization of almond germplasm: genetic diversity among cultivated genotypes and related wild species of almond, and its relationships with agronomic traits. *Euphytica,* **156**, 327–344.

Sosinski, B., Gannavarapu, M., Hager, L. D., Beck, L. E., King, G. J., Ryder, C. D., Rajapakse, S., Baird, W. V., Ballard, R. E., Abbott, A. G. (2000): Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.,* **101**, 421–428.

Sutherland, B. G., Robbins, T. P., Tobutt, K. R. (2004): Primers amplifying a range of *Prunus S*-alleles. *Plant Breeding,* **123**, 582–584.

Tamura, M., Ushijima, K., Sassa, H., Hirano, H., Tao, R., Gradziel, T. M., Dandekar, A. M. (2000): Identification of self-incompatibility genotypes of almond by allele-specific PCR analysis. *Theor. Appl. Genet.,* **101**, 344–349.

Testolin, R., Marrazzo, T., Cipriani, G., Quarta, R., Verde, I., Dettori, M. T., Pancaldi, M., Sansavini, S. (2000): Microsatellite DNA in peach (*Prunus persica* L. Batsch) and its use in fingerprinting and testing the genetic origin of cultivars. *Genome,* **43**, 512–520.

Testolin, R., Messina, R., Lain, O., Marrazzo, M. T., Huang, W. G., Cipriani, G. (2004): Microsatellites isolated in almond from an AC-repeat enriched library. *Mol. Ecol. Notes,* **4**, 459–461.

Tufts, W. P., Philp, G. L. (1922): Almond pollination. *California Agricultural Experimental Station Bulletin,* **346**, 1–35.

Ushijima, K., Sassa, H., Dandekar, A. M., Gradziel, T. M., Tao, R., Hirano, H. (2003): Structural and transcriptional analysis of the self-incompatibility locus of almond: Identification of a pollen-expressed F-box gene with haplotype-specific polymorphism. *Plant Cell,* **15**, 771–781.

Vezvaei, A., Hancock, T. W., Giles, L. C., Clarke, G. R., Jackson, J. F. (1995): Inheritance and linkage of isozyme loci in almond. *Theor. Appl. Genet.,* **91**, 432–438.

Viruel, M. A. (1995): *Construcción de un mapa genético con marcadores moleculares en almendro (Prunus amygdalus Batsch.). Estudio de la variabilidad intraespecífica y aplicación a la identificación varietal.* [Construction of a genetic map using molecular markers in almond (*Prunus amygalus* Batsch.). Study on intraspecific variability and its application in variety identification.] Unpublished Doctoral Thesis, Universitat de Barcelona, Barcelona, Spain.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. v. d., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., Zabeau, M. (1995): AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.,* **23**, 4407–4414.

Wang, Y., Georgi, L. L., Zhebentyayeva, T. N., Reighard, G. L., Scorza, R., Abbott, A. G. (2002): High-throughput targeted SSR marker development in peach (*Prunus persica*). *Genome,* **45**, 319–328.

Wu, S.-B., Wirthensohn, M., Hunt, P., Gibson, J., Sedgley, M. (2008): High resolution melting analysis of almond SNPs derived from ESTs. *Theor. Appl. Genet.,* **118**, 1–14.

Wünsch, A., Hormaza, J. I. (2002): Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. *Euphytica,* **125**, 59–67.

Xie, H., Sui, Y., Chang, F.-Q., Xu, Y., Ma, R.-C. (2006): SSR allelic variation in almond (*Prunus dulcis* Mill.). *Theor. Appl. Genet.,* **112**, 366–372.

Yamamoto, T., Mochida, K., Imai, T., Shi, Y. Z., Ogiwara, I., Hayashi, T. (2002): Microsatellite markers in peach [*Prunus persica* (L.) Batsch] derived from an enriched genomic and cDNA libraries. *Mol. Ecol. Notes,* **2**, 298–301.

Zeinalabedini, M., Khayam-Nekoui, M., Grigorian, V., Gradziel, T. M., Martínez-Gómez, P. (2010): The origin and dissemination of the cultivated almond as determined by nuclear and chloroplast SSR marker analysis. *Sci. Hortic.-Amsterdam,* **125**, 593–601.

Zeinalabedini, M., Majourhat, K., Khayam-Nekoui, M., Grigorian, V., Torchi, M., Dicenta, F., Martínez-Gómez, P. (2008): Comparison of the use of morphological, protein and DNA markers in the genetic characterization of Iranian wild *Prunus* species. *Sci. Hortic.-Amsterdam,* **116**, 80–88.

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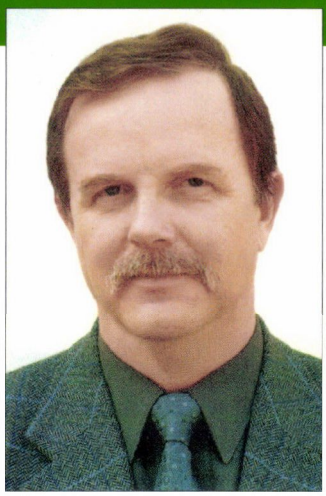

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